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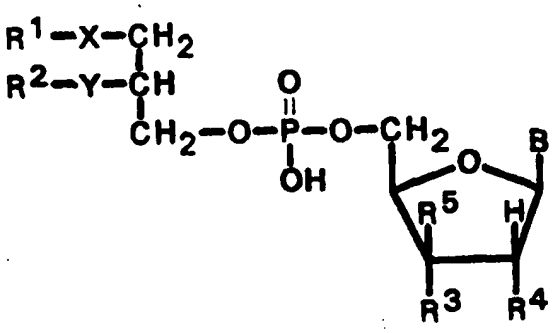
(57) Abstract

The present invention is directed to new nucleoside monophosphate derivatives of lipid ester residues of general formula (I), wherein R^1 represents an optionally substituted alkyl chain having 1-20 carbon atoms; R^2 represents hydrogen, an optionally substituted alkyl chain having 1-20 carbon atoms; R^3, R^4 and R^5 represent hydrogen, hydroxy, azido, amino, cyano, or halogen; X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group; Y represents a valence dash, an oxygen or sulfur atom; B represents a purine and/or pyrimidine base; with the proviso that at least one of the residues R^3 or R^5 is hydrogen; to their tautomers and their physiologically acceptable salts of inorganic and organic acids and/or bases, as well as to processes for their preparation, and to drugs containing said compounds.



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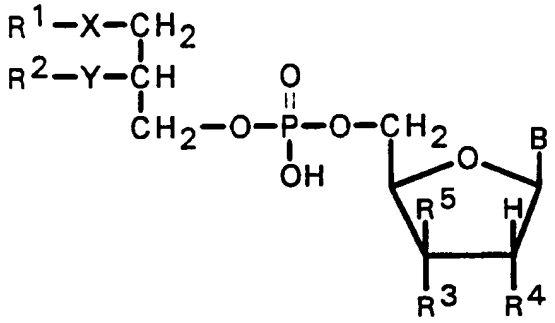
New Lipid Esters of Nucleoside Monophosphates and Their Use as Immunosuppressive Drugs

The present invention is directed to new nucleoside monophosphate derivatives of lipid ester residues of general formula (I)



(I),

wherein

R^1 may be a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfinyl, or C_1-C_6 alkylsulfonyl groups;

R^2 may be hydrogen, a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, or C_1-C_6 alkylsulfonyl groups;

R^3 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

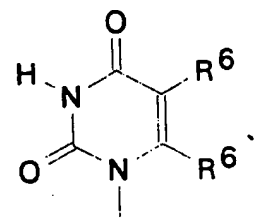
R^4 represents hydroxy, azido, amino, cyano, or halogen;

R^5 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

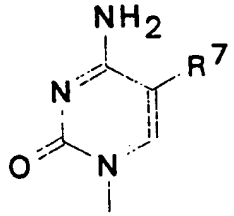
X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group;

Y is a valence dash, an oxygen or sulfur atom;

B represents a purine and/or pyrimidine base of formula III(a-d)



(IIIa)



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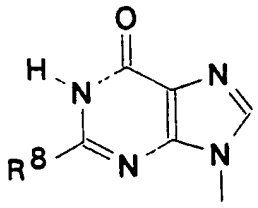

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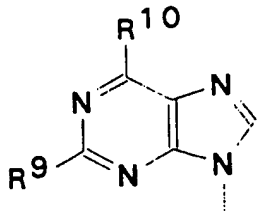


(IIId)

wherein

R^6 may be hydrogen; an alkyl chain having 1-6 carbon atoms, which may be substituted by halogen; an alkenyl and/or alkinyl residue having 2-6 carbon atoms, optionally substituted by halogen; or halogen;

$R^{6'}$ may be a hydrogen atom or a benzyl or phenylthio residue;

R^7 may be hydrogen; an alkyl chain having 1-6 carbon atoms, which may be substituted by halogen; or halogen;

R^8 may be hydrogen, an alkyl chain having 1-6 carbon atoms, halogen, or a hydroxy or an amino group;

R^9 may be hydrogen, an amino group or a halogen atom; and

R^{10} may be hydrogen, halogen, mercapto, hydroxy, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy-C_2-C_6 alkyl, and/or C_3-C_6 cycloalkyl, aryl, hetaryl, aralkyl, or hetarylalkyl groups, optionally substituted at the aryl or hetaryl residue by one or more mercapto, hydroxy, C_1-C_6 alkoxy, or C_1-C_6 alkyl groups or halogen; or C_2-C_6 alkenyl optionally substituted by mono- or dialkyl or alkoxy groups;

with the proviso that at least one of the residues R^3 or R^5 is hydrogen;

to their tautomers and their physiologically acceptable salts of inorganic and organic acids and/or bases, as well as to processes for their preparation, and to drugs containing said compounds.

As these compounds of general formula I contain asymmetric carbon atoms, the invention is likewise directed to all the optically active forms and racemic mixtures of said compounds.

J. Biol. Chem. 265, 6112 (1990), and EP 0,350,287 describe preparation and use of liponucleotides as antiviral drugs. Therein, however, only dimyristoylphosphati-

dyl and dipalmitoylphosphatidyl residues coupled to familiar nucleosides such as AZT (azidothymidine) and ddC (2',3'-dideoxycytidine) have been examined and synthesized, including their fatty acid ester structure.

J. Med. Chem. 33, 1380 (1990), describes nucleoside conjugates of thioether lipides with cytidine diphosphate, which have antitumor activity and might find use in oncology. Chem. Pharm. Bull. 36, 209 (1988), describes 5'-(3-sn-phosphatidyl)nucleosides having antileukemic activity, as well as their enzymatic synthesis from the corresponding nucleosides and phosphocholines in the presence of phospholipase D with transferase activity. Similarly, J. Med. Chem. 34, 1408 (1991), describes nucleoside conjugates having anti-HIV 1 activity, which are substituted by methoxy or ethoxy in sn-2 position of the lipid portion. The patent application WO 92/03462 describes thioether lipid conjugates having antiviral activity, particularly for treating HIV infections.

The compounds of the present invention have valuable pharmacological properties. In particular, they are suitable in therapy and prophylaxis of malignant tumors such as malignancies, neoplasms, carcinomas, sarcomas, or leukemias in tumor therapy. In addition, the compounds exhibit immunosuppressive activity and therefore, they may be employed in the therapy of organ-specific or generalized auto-immune diseases such as rheumatoid arthritis, systemic lupus erythematosus, chronic graft vs. host disease, multiple sclerosis, etc., or in preventing allogenic or semiallogenic graft rejection, e.g., kidneys, liver, lungs,

heart, etc.. Furthermore, the compounds have antiviral, anti-retroviral or anti-oncogenic activity and thus, are also suitable in prophylaxis and therapy of viral and oncogenic-induced/caused diseases (such as AIDS etc.). Compared to compounds hitherto employed in treatment of malign tumors, the compounds according to the invention have enhanced efficacy or lower toxicity and thus, have a wider therapeutic range. For this reason, they are advantageous in that the administration of drugs containing these compounds may be conducted continuously over a prolonged period of time, and withdrawal of the preparation or intermittent administration, which frequently has been routine with cytostatic agents hitherto employed in tumor therapy or, due to their undesirable side-effects, has been necessary, can be avoided.

The compounds according to the invention do not suffer from these drawbacks. Their action is immunosuppressive or antitumoral, without being unspecifically cytotoxic in pharmacologically relevant doses.

Similarly, the compounds of the present invention and their pharmaceutical formulations may be employed in combination with other drugs for the treatment and prophylaxis of the diseases mentioned above. Examples of these further drugs involve agents such as, e.g., mitosis inhibitors such as colchicine, mitopodozid, vinblastine, alkylating cytostatic agents such as cyclophosphamide, melphalan, myleran or cisplatin, antimetabolites such as folic acid antagonists (methotrexate) and antagonists of purine and pyrimidine bases (mercaptopurine, 5-fluorouridine, cytarabin), cytostatically active antibiotics such as anthracyclines (e.g., doxorubicin, daunorubicin), hormones such as fosfestrol, tamoxifen, other cytostatically/cytotoxically active chemotherapeutic agents and other immunosuppressive drugs (such as cyclosporines, FK 506, rapamycines, desoxyspergualin, etc.).

Above all, possible salts of the compounds of general formula I are the alkali, alkaline earth and ammonium salts of the phosphate group. Preferred as the alkali salts are lithium, sodium and potassium salts. Possible as the alkaline earth salts are magnesium and calcium, in particular. According to the invention, ammonium salts are understood to be those containing the ammonium ion which may be substituted up to four times by alkyl residues having 1-4 carbon atoms, and/or aralkyl residues, preferably benzyl residues. Here, the substituents may be the same or different.

The compounds of general formula I may contain basic groups, particularly amino groups, which may be converted to acid addition salts by suitable inorganic or organic acids. To this end, possible as the acids are, in particular: hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, fumaric acid, succinic acid, tartaric acid, citric acid, lactic acid, maleic acid, or methanesulfonic acid.

In the general formula I, R^1 preferably represents a straight-chain C_8-C_{15} alkyl group which may be further substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group. More specifically, R^1 represents a nonyl, decyl, undecyl, dodecyl, tridecyl, or tetradecyl group. Preferably, methoxy, ethoxy, butoxy, and hexyloxy groups are possible as the C_1-C_6 alkoxy substituents of R^1. In case R^1 is substituted by a C_1-C_6 alkylmercapto residue, this is understood to be the methylmercapto, ethylmercapto, propylmercapto, butylmercapto, and hexylmercapto residue, in particular.

Preferably, R^2 represents a straight-chain C_8-C_{15} alkyl group which may be further substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group. More speci-

fically, R^2 represents an octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, or tetradecyl group. Preferably, methoxy, ethoxy, propoxy, butoxy, and hexyloxy groups are preferable as the C_1-C_6 alkoxy substituents of R^2. In case R^1 is substituted by a C_1-C_6 alkylmercapto residue, this is understood to be the methylmercapto, ethylmercapto, butylmercapto, and hexylmercapto residue, in particular.

Preferably, X is sulfur, sulfinyl or sulfonyl, and Y is oxygen.

Similarly, compounds are preferred, wherein X and Y represent a valence dash, R^2 is hydrogen, and R^1 represents a C_1-C_{20} alkyl chain optionally substituted by C_1-C_6 alkoxy or C_1-C_6 alkylmercapto.

Preferably, R^5 represents hydrogen, azido, cyano or halogen, such as fluorine, chlorine or bromine.

Preferably, each R^3 and R^4 represent a hydroxy or a cyano or azido group, or a halogen atom, such as fluorine, chlorine, bromine or iodine, wherein the residues may be the same or different.

Particularly preferred are compounds, wherein R^5 represents a hydrogen atom and R^3 and R^4 are hydroxy, cyano, azido or fluorine.

In the bases of general formula (III) the residues R^6 and R^7 preferably represent a hydrogen atom, a methyl, trifluoromethyl, ethyl, propyl, or butyl residue, or a halogen atom, such as fluorine, chlorine, bromine or iodine, as well as an alkenyl and/or alkinyl group which may be substituted by halogen.

Particularly preferred for R^6 and R^7 is a hydrogen atom, the methyl, trifluoromethyl or ethyl residues, and a fluorine, chlorine or bromine atom, and/or the vinyl, propenyl, ethinyl or propinyl residues optionally substituted by halogen.

Preferably, the residue R^8 is a hydrogen atom, a methyl, ethyl, propyl, or butyl residue, an amino group or a halogen atom such as fluorine, chlorine bromine or iodine, preferably chlorine or bromine.

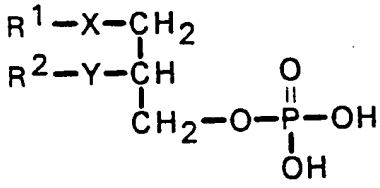
Preferably, R^{10} represents a hydrogen, fluorine, chlorine or bromine atom, a C_1-C_6 alkoxy group, more specifically a methoxy, ethoxy, propoxy, butoxy, or hexyloxy group, a mercapto residue, a C_1-C_6 alkylmercapto group, more specifically a methylmercapto, ethylmercapto, butylmercapto, or hexylmercapto group, or an amino group which may be mono- or disubstituted by a C_1-C_6 alkyl group, such as the methyl, ethyl, butyl or hexyl groups, by a hydroxy-C_2-C_6 alkyl group, such as the hydroxyethyl, hydroxypropyl, hydroxybutyl, or hydroxyhexyl groups, by a C_3-C_6 cycloalkyl residue, such as the cyclopropyl, cyclopentyl or cyclohexyl residues, by aryl, preferably phenyl, by an aralkyl residue, such as, in particular, benzyl optionally substituted by one or more hydroxy or methoxy groups, by C_1-C_6 alkyl groups, such as the methyl, ethyl, propyl, butyl, or hexyl groups, or by halogen atoms, such as fluorine, chlorine or bromine. Similarly, the amino group may be substituted by a hetarylalkyl or hetaryl residue such as, in particular, the thienyl, furyl or pyridyl residues, for example. Preferably, the hetaryl residue is understood to be the thienylmethyl, furylmethyl or pyridylmethyl residue.

Preferably, the following nucleosides are suitable as the coupling components to prepare the lipid-nucleotide conjugates of formula (I):

6-Mercaptopurine-9-β-D-ribofuranoside
5-Fluorouridine
Inosine
5-Methyluridine
2',3'-Didesoxy-2',3'-difluorothymidine
5-Chlorouridine
5-Trifluoromethyluridine
5-Ethinyluridine
5-Ethinylcytidine
5-Prop-1-enyluridine
5-Prop-2-enyluridine
Adenosine
Guanosine
2,6-Diaminopurine-9-β-D-ribofuranoside
2-Amino-6-mercaptopurine-9-ß-D-ribofuranoside
2-Amino-6-mercaptomethylpurine-9-ß-D-ribofuranoside
2-Amino-6-chloropurine-9-ß-D-ribofuranoside
2'-Desoxy-2'-aminoadenosine
2'-Desoxy-2'-azidoadenosine
2'-Desoxy-2'-azidocytidine
2'-Desoxy-5-fluorouridine
2-Chloroadenosine
2-Bromoadenosine
3'-Desoxy-3'-fluoroadenosine
6-Methylmercaptopurine-9-ß-D-ribofuranoside
2-Fluoroadenosine
2-Fluoro-2'-desoxyadenosine

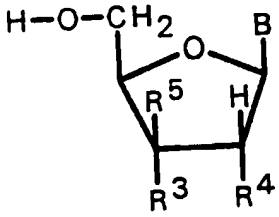
The compounds of general formula (I) may be prepared by

1. reacting a compound of general formula V



(V),

wherein R^1, R^2, X and Y have the meanings as indicated, with a compound of general formula VI

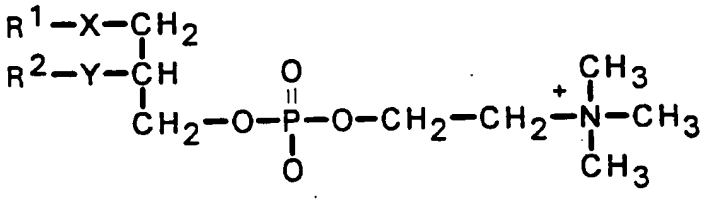


(VI),

wherin R^3, R^4, R^5 and B have the above-mentioned meanings, or represent a hydroxy group protected by an oxygen protecting group familiar to the artisan,

in the presence of an activating acid chloride, such as 2,4,6-triisopropylbenzenesulfonic acid chloride, and a tertiary nitrogen base, e.g., pyridine or lutidine, in an inert solvent, such as toluene, or immediately in anhydrous pyridine, and optionally, subsequent to hydrolysis, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry, or

2. reacting a compound of general formula VII



(VII),

wherein R^1, R^2, X and Y have the above-mentioned meanings, with a compound of general formula VI, wherein R^3, R^4, R^5 and B have the above-mentioned meanings, in the presence of phospholipase D from *Streptomyces* in an inert solvent such as chloroform, in the presence of a suitable buffer, and optionally, subsequent to reaction, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry.

The preparation of the compounds of general formula V and VII is performed in analogy to Lipids 22, 947 (1987), and J. Med. Chem. 34, 1377 (1991).

The preparation of the compounds of general formula VI is described, e.g., in EP-A-0,286,028 and WO 90/08147. Some of the included nucleosides are commercially available compounds.

Compounds similar to formula I are described in EP-A-0,350,287. Therein, the corresponding 1,2-diesters of glycerol are described.

The drugs containing compounds of formula I for the treatment of viral infections may be applied in liquid or solid forms on the intestinal or parenteral route. Here, the common application forms are possible, such as tablets, capsules, coated tablets, syrups, solutions, or suspensions. Preferably, water is used as the injection medium, containing additives such as stabilizers, solubilizers and buffers as are common with injection solutions. Such additives are, e.g., tartrate and citrate buffers, ethanol, complexing agents such as ethylenediaminetetraacetic acid and its non-toxic salts, high-molecular polymers such as liquid polyethylene oxide for viscosity control. Liquid vehicles for injection solutions need to be sterile and are filled in ampoules, preferably. Solid car-

riers are, for example, starch, lactose, mannitol, methylcellulose, talc, highly dispersed silicic acids, higher-molecular fatty acids such as stearic acid, gelatine, agar-agar, calcium phosphate, magnesium stearate, animal and plant fats, solid high-molecular polymers such as polyethylene glycol, etc.. If desired, formulations suitable for oral application may include flavorings or sweeteners.

The dosage may depend on various factors such as mode of application, species, age, or individual condition. Conventionally, the compounds according to the invention are applied in amounts of 0.1-100 mg, preferably 0.2-80 mg per day and per kg of body weight. It is preferred to divide the daily dose into 2-5 applications, with tablets having an active ingredient content of 0.5-500 mg being administered with each application. Similarly, the tablets may have sustained release, reducing the number of applications to 1-3 per day. The active ingredient content of sustained-release tablets may be 2-1000 mg. The active ingredient may also be administered by continuous infusions, where amounts of 5-1000 mg per day are normally sufficient.

In addition to the compounds mentioned in the examples, the following compounds of formula I are possible in the meaning of the present invention:

1. (5-Chlorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
2. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
3. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
4. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

5. (5-Prop-1-enyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
6. (5-Ethinylcytidine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
7. (2-Amino-6-mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
8. (2,6-Diaminopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
9. (5-Prop-2-enyluridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
10. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
11. (5-Chlorouridine)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
12. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylsulfonyl-2-decyloxy)propyl ester
13. (5-Fluorouridine)-5'-phosphoric acid (3-dodecyloxy-2-decyloxy)propyl ester
14. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecyloxy-2-decyloxy)propyl ester
15. (5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decylmercapto)propyl ester
16. (5-Fluorouridine)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
17. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
18. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-undecylmercapto-2-undecyloxy)propyl ester
19. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-decylmercapto-2-dodecyloxy)propyl ester
20. (5-Fluorouridine)-5'-phosphoric acid (3-undecylmercapto-2-dodecyloxy)propyl ester
21. (5-Trifluoromethyluridine)-5'-phosphoric acid (3-undecylmercapto-2-decyloxy)propyl ester
22. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-tetradecylmercapto-2-decyloxy)propyl ester

23. (5-Fluorouridine)-5'-phosphoric acid (3-tridecylmercapto-2-decyloxy)propyl ester
24. (2-Fluoroadenosine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
25. (2-Desoxy-2-fluoroadenosine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester
26. (6-Mercaptopurine)-9-ß-D-ribofuranoside)-5'-phosphoric acid dodecyl ester
27. (5-Fluorouridine)-5'-phosphoric acid hexadecyl ester
28. (5-Trifluoromethyluridine)-5'-phosphoric acid eicosyl ester
29. (5-Fluorouridine)-5'-phosphoric acid dodecyl ester
30. (6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid dodecyl ester

Example 1

(5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

3.6 g (6.1 mmoles) of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was treated twice with 30 ml of anhydrous pyridine and concentrated by evaporation. The residue was dissolved in 30 ml of anhydrous pyridine, treated with 2.76 g (9.1 mmoles) of 2,4,6-triisopropylbenzenesulfonic acid chloride under nitrogen and stirred at room temperature for 30 minutes. Then, 1.60 g (6.1 mmoles) of 5-fluorouridine (Fluka) was added, and the charge was allowed to stand under N_2 for 24 hours.

Hydrolysis was performed using 15 ml of water, the mixture was stirred for another 2 hours at room temperature, freed from solvent under vacuum, and stripped twice using a small amount of toluene. The residue was purified by column chromatography on LiChroprep® RP-18 with a linear

gradient of methanol/water 7/1 to methanol as the eluant. The yield is 3.1 g (69% of theoretical amount); oil. R_f = 0.24 (CH_2Cl_2/MeOH 8/2); R_f = 0.55 (CH_2Cl_2/MeOH/H_2O 6.5/2.5/0.4) on Merck 5715 TLC plates, silica gel 60 F.

The phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was prepared as described in WO 92/03462.

Example 2

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester

6.2 g (12.5 mmoles) of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester was treated with 5.7 g (18.75 mmoles) of 2,4,6-triisopropylbenzenesulfonic acid chloride as described in Example 1 and subsequently with 3.55 g (11.25 mmoles) of 6-mercaptopurine-9-ß-D-ribofuranoside and after 24 hours, this was hydrolyzed with water.

Then, 2.85 g of calcium acetate in 15 ml of water was slowly dropped therein, precipitating the crude calcium salt of the conjugate. After prolonged stirring the precipitate with acetone (1/10), 6 g of an amorphous crude product was obtained, having 72 area -% according to HPLC.

The calcium salt was suspended in 350 ml of methanol, treated with 150 g of Amberlite IR 120 in the Na^+ form and stirred for 2 days.

Thereafter, the ion exchanger was removed, the filtrate was evaporated, and the residue was purified by column chromatography on LiChroprep® RP-18 with a linear gradient of methanol/water 5/1 to 9/1. The fractions containing

product were evaporated in a vacuum, and the residue was stirred with acetone and dried. Yield: 3.52 g (41% of theoretical amount).

DC: R_f = 0.45 (isopropanol/butyl acetate/conc. ammonia/water 50/30/5/15).

Example 3

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 2, 41.4 g of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester in 400 ml of anhydrous pyridine was reacted with 42.9 g of 2,4,6-triisopropylbenzenesulfonic acid chloride and subsequently with 23.7 g of 6-mercaptopurine-9-ß-D-ribofuranoside. The crude calcium salt which was filtered by suction after hydrolysis and precipitation with 25 g of calcium acetate in 160 ml of water, was distributed between 500 ml of MTB and 250 ml of 2N HCl and stirred until completely dissolved in the organic phase. The organic phase was separated, washed with saturated sodium chloride solution and concentrated in a rotary evaporator. The residue was applied onto 80 g of LiChroprep RP-18 (treat MTB solution of crude product with RP-18, evaporate and dry), and separated portion by portion in a pre-column on RP-18. Each time, a mixture of 3.7 l of methanol, 400 ml of water, 3 ml of glacial acetic acid, and 2 g of sodium acetate served as the eluant. The fractions containing product were combined, the desired compound was precipitated by adding 20 g of calcium acetate in 100 ml of water and filtered by suction. Yield: 32 g (43% of theoretical amount).

The calcium salt was suspended in 250 ml of MTB, extracted with 80 ml of 2N HCl by shaking, and the organic phase was washed twice with saturated sodium chloride solution. Following removal of the solvent, the residue was dissolved in 200 ml of toluene and adjusted to pH 7 against a Friscolyt electrode with 30% sodium methylate solution. The sodium salt was precipitated by stirring into 200 ml of acetone, filtered by suction and dried in a vacuum drying oven. Yield: 29 g (37% of theoretical amount).

R_f value: 0.18 (Silica gel; eluant: isopropanol/butyl acetate/water/conc. ammonia 50/30/15/5).

Example 4

(6-Mercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 3, the crude conjugate was prepared from 40 g of 6-mercaptopurine-9-ß-D-ribofuranoside. The crude product was purified by column chromatography using 8 g each time, on a column with DIOL phase (diameter 4 cm; length 25 cm) (detection at 254 nm; eluant: methanol/MTB 10/4). The applied sample had clearly dissolved in the eluant. The product-containing fractions of the different separations were combined, evaporated and precipitated as the sodium salt from toluene and acetone as in Example 3. Yield: 64.5 g (51% of theoretical amount).

R_f value: 0.85 (DIOL phase; eluant: methanol).

Example 5

(6-Methylmercaptopurine-9-ß-D-ribofuranoside)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 1, 14.9 g of 6-methylmercaptopurine-9-ß-D-ribofuranoside (50 mmoles) were reacted with the mixed anhydride prepared from 27.3 g of phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester and 25 g of 2,4,6-triisopropylbenzenesulfonic acid chloride in 250 ml of anhydrous pyridine, hydrolyzed and concentrated by evaporation. Analogous to Example 3, the crude product (HPLC: 67 area %) was purified by chromatography on RP-18, precipitated as the calcium salt, and converted to the sodium salt. Yield: 15.2 g (38% of theoretical amount).

R_f value: 0.22 (Silica gel; eluant: isopropanol/butyl acetate/water/conc. ammonia 50/30/15/5).

Example 6

(5-Fluorouridine)-5'-phosphoric acid (3-dodecylmercapto-2-decyloxy)propyl ester sodium salt

Analogous to Example 1, 50 g of 5-fluorouridine was converted to the crude conjugate, precipitated as the calcium salt as described in Example 3 and subsequent to conversion to the free acid, was purified as the crude product by chromatography, analogous to Example 4, on a DIOL phase using methanol/MTB 10/4 as the eluant. The sodium salt prepared as in Example 3 was isolated in a yield of 69%.

R_f value: 0.35 (DIOL plates; eluant: methanol/MTB 10/4).

Example 7

IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6- MP), 6-Mercaptopurinribosid, BM 92.0729 and Doxorubicin in CFU-E and CFU-GM assays

This table shows the IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6-MP) and 6-Mercaptopurinribosid in comparison to the 6-Mercaptopurinribosid ether lipid conjugate BM 92.0729 for in vitro cytotoxicity on murine bone marrow stern cells, including colony-forming units/erythrocytes (CFU-E) and colony-forming units/granulocytes-macrophages (CFU-GM). The cytostatic/cytotoxic compound Doxorubicin was also included as reference substance. All compounds were tested in 3-6 different experiments concentration dependently with,at least, doublicate or triplicate inclubations per concentration tested.

As can be seen from the results, BM 92.0700 is much better tolerated by the bone marrow stern cells compared to all other compounds tested, in particular, in comparison to 6-Mercaptopurinribosid.

IC_{50} values (µg/ml) for Azathioprine, 6-Mercaptopurine (6-MP), 6-Mercaptopurinribosid, BM 92.0729 and Doxorubicin in CFU-E and CFU-GM assays.[a]

Compound	CFU-E				CFU-GM			
Azathioprine	0.0004	±	0.0001	(4)	0.0043	±	0.0019	(3)
6-MP	0.0003	±	0.0001	(4)	0.0023	±	0.00009	(3)
6-MP-Ribosid	0.0003	±	0.0001	(4)	0.0023	±	0.00013	(3)
BM 92.0729	0.056	±	0.013	(5)	0.247	±	0.044	(6)
Doxorubicin	0.0017	±	0.0005	(4)	0.050	±	0.004	(4)

[a] Mean ± SEM; n, number of different experiments.

Example 8

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine and 6-Mercaptopurinribosid in female Balb/c mice: Day + 4 (Exp. 930740)

Exp. 930740 shows the bone marrow toxicity of BM 92.0792, Azathioprine, , 6-Mercaptopurine and 6-Mercaptopurinribosid in vivo in female Balb/c mice which were treated once daily p.o. for four consecutive days (day 0-day +3). The animals were killed on day +4 and bone marrow cellularity (cells/fernur) was determined. The results indicate no bone marrow toxicity for the 6-Mercaptopurinribosid ether lipid conjugate BM 92.0729 up to the highest dose tested, i.e. 100 $mg \cdot kg^{-1} \cdot day^{-1}$ which correspondenz on a molar basis with 30 $mg \cdot kg^{-1} \cdot day^{-1}$ of 6-Mercaptopurinribosid . This latter compond shows, in contrast to the ether lipid conjugate BM 92.0792, clearly a dose-dependent reduciton in one marrow cellularity. The same finding was obtained for the other substances, including Azathioprine and 6-Mercaptopurine .

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine and 6-Mercaptopurinribosid in female Balb/c mice: Day + 4 (Exp. 930740)

Compound	Dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cells/femur (10^6)	
Control (0,5% Tylose)	-	15.9 ± 1.4 (8)	[a]
Azathioprine	10	11.6 ± 0.4 (9)	*
Azathioprine	30	9.6 ± 0.9 (9)	**
6-Mercaptopurine	10	13.0 ± 1.5 (8)	
6-Mercaptopurine	30	6.5 ± 0.7 (9)	**
6-Mercaptopurinribosid	10	12.6 ± 0.5 (9)	**
6-Mercaptopurinribosid	30	9.3 ± 0.5 (9)	**
BM 92.0729	30	15.4 ± 0.9 (9)	
BM 92.0729	100	13.0 ± 0.6 (9)	

[a] mean + SEM; Treatment once daily p.o., day 0-day+3
Sacrifice on day +4

* $p \leq 0.05$
** $p \leq 0.01$
} Mann-Whitney-test

Example 9

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine, 6-Mercaptopurinribosid and Cyclosporin A in female Balb/c mice: Day + 4 (Exp. 940026).

Exp. 940026 is an experiment which was aimed at reproducing the results obtained in Exp. 930740 (Example 8). In this experiment Cyclosporin A was included as a reference compound, too. The outcome of the Exp. 940026 confirmed the results obtained in Exp. 930740 in vivo.

Bone marrow toxicity of BM 92.0729, Azathioprine, 6-Mercaptopurine, 6-Mercaptopurinribosid and Cyclosporin A in female Balb/c mice: Day + 4 (Exp. 940026)

Compound	Dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cells/femur (10^6)	
Control (0,5% Tylose)	-	15.6 ± 0.8 (10)	a
Azathioprine	10	11.1 ± 0.6 (10)	**
Azathioprine	30	9.1 ± 0.5 (10)	**
6-Mercaptopurine	10	10.9 ± 0.9 (10)	*
6-Mercaptopurine	30	6.2 ± 0.5 (10)	**
6-Mercaptopurinribosid	10	13.7 ± 1.4 (10)	*
6-Mercaptopurinribosid	30	8.4 ± 0.4 (10)	**
BM 92.0729	30	14.3 ± 0.5 (10)	
BM 92.0729	100	13.0 ± 0.4 (10)	
Cyclosporin A	5	13.1 ± 0.4 (10)	
Cyclosporin A	10	7.6 ± 1.4 (10)	**

[a] mean + SEM; Treatment once daily p.o., day 0-day+3
Sacrifice on day +4

* $p \leq 0.05$
** $p \leq 0.01$
} Mann-Whitney-test

Example 10

Bone marrow toxicity (µM) of BM 92.0700 and 5-FU in CFU-E and CFU-GM assays.

The table shown in Encl. 4 gives the mean IC_{50} values for 5-Fluorouridine (5-FU) and the 5-FU ether lipid conjugate BM 92.0700 for bone marrow toxicity in vitro in CFU-E and CFU-GM assays. For assay conditions, please refer to description of Encl. 1.

The data indicate that the ether lipid conjugate of 5-Fluorouridine BM 92.0700 is 610 times and 238 times less toxic on erythrocyte and granulocyte/macrophage bone marrow stem cells, respectively, compared to 5-FU itself.

Bone marrow toxicity (μM) of BM 92.0700 and 5-FU in CFU-E and CFU-GM assays

Compound	CFU-E[a]		CFU-GM[a]	
BM 92.0700	0.372 (3)	610 x	1.178 (7)	238 x
5-FU	0.00061 (3)		0.00496 (10)	

[a] **Mean; n, number of experiments**

Example 11

Influence of the 5-FU ether lipid conjugate BM 92.0700 (Fig. 1) and of 5-FU (Fig. 2) on the L 1210 leukemia in vivo: Survival time.

Mice were inoculated with L 1210 leukemia cells on day 0 (n = 10 animals/group) and were then treated once daily i.p. from day 0 (+1h) - day +41 (6 weeks) with the weekly cycles indicated on Encl. 5 and 6, respectively.

From the survival curves of the control and treatment groups shown on Encl. 6 it is obvious, that 5-FU has, as reported in the literature, a very narrow dose-efficacy profile, i.e. icreasing the dose, for example from 2 x 10/5 x 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ to 2 x 10/5 x 0.3 $mg \cdot kg^{-1} \cdot day^{-1}$ or to even higher doses lead to reduced survival rates.

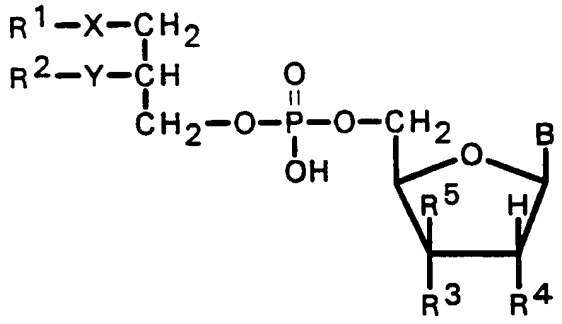
In contrast, with the 5-FU ether lipid conjugate BM 92.0700 a clear dose-dependent increase in survival time was obtained compared to control I and II (Fig. 1) indicating

that equimolar doses of BM 92.0700 are clearly more effective in this leukemia model compared to the standard compound 5-FU.

Taken into consideration that BM 92.0700 is more effective (Fig. 1 and 2) and much less toxic on bone marrow cells it can be concluded that BM 92.0700 has a much higher therapeutic index/ratio compared to the standard cytostatic 5-FU.

C L A I M S

1. Nucleoside monophosphate derivatives of formula (I)



(I),

wherein

R^1 may be a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfinyl, or C_1-C_6 alkylsulfonyl groups;

R^2 may be hydrogen, a straight-chain or branched, saturated or unsaturated alkyl chain having 1-20 carbon atoms, optionally mono- or polysubstituted by halogen, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, C_1-C_6 alkoxycarbonyl, or C_1-C_6 alkylsulfonyl groups;

R^3 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

R^4 represents hydroxy, azido, amino, cyano, or halogen;

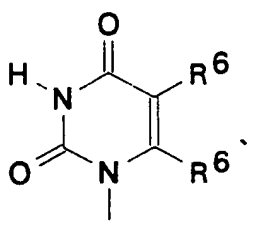
R^5 represents hydrogen, hydroxy, azido, amino, cyano, or halogen;

X represents a valence dash, oxygen, sulfur, a sulfinyl or sulfonyl group;

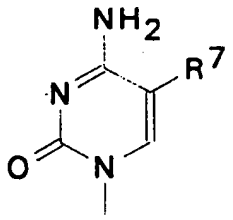
Y is a valence dash, an oxygen or sulfur atom;

B represents a purine and/or pyrimidine base of formula III(a-d)

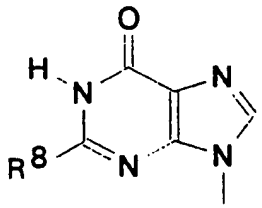
wherein



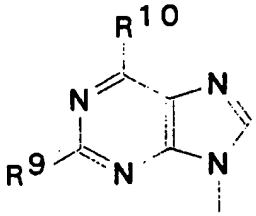
(IIIa)



(IIIb)



(IIIc)



(IIId)

R^6 may be hydrogen; an alkyl chain having 1-4 carbon atoms, which may be substituted by halogen; an alkenyl and/or alkinyl residue having 2-6 carbon atoms, optionally substituted by halogen; or halogen;

$R^{6'}$ may be a hydrogen atom or a benzyl or phenylthio residue;

R^7 may be hydrogen; an alkyl chain having 1-4 carbon atoms, which may be substituted by halogen; or halogen;

R^8 may be hydrogen, an alkyl chain having 1-4 carbon atoms, halogen, or a hydroxy or an amino group;

R^9 may be hydrogen, an amino group or a halogen atom; and

R^{10} may be hydrogen, halogen, mercapto, hydroxy, C_1-C_6 alkoxy, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy-C_2-C_6 alkyl, and/or C_3-C_6 cycloalkyl, aryl, hetaryl, aralkyl, or hetarylalkyl groups, optionally substituted at the aryl or hetaryl residue by one or more mercapto, hydroxy, C_1-C_6 alkoxy, or C_1-C_6 alkyl groups or halogen; or C_2-C_6 alkenyl optionally substituted by mono- or dialkyl or alkoxy groups;

with the proviso that at least one of the residues R^3 or R^5 is hydrogen;

to their tautomers, their optically active forms and racemic mixtures, and to their physiologically acceptable salts of inorganic and organic acids or bases.

2. Nucleoside monophosphate derivatives of formula I according to claim 1, characterized in that R^1 represents a straight-chain C_8-C_{15} alkyl group which may be substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group.

3. Nucleoside monophosphate derivatives of formula I according to claim 1 or 2, characterized in that

R^2 represents a straight-chain C_8-C_{15} alkyl group which may further be substituted by a C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto group.

4. Nucleoside monophosphate derivatives of formula I according to one of claims 1-3, characterized in that X represents sulfur, sulfinyl or sulfonyl, and Y represents oxygen.

5. Nucleoside monophosphate derivatives of formula I according to one of claims 1-3, characterized in that X and Y represent a valence dash, R^2 is hydrogen, and R^1 represents a C_1-C_{20} alkyl chain which optionally may be substituted by C_1-C_6 alkoxy or a C_1-C_6 alkylmercapto.

6. Nucleoside monophosphate derivatives of formula I according to one of claims 1-5, characterized in that R^5 represents hydrogen, azido, cyano, or halogen.

7. Nucleoside monophosphate derivatives of formula I according to one of claims 1-6, characterized in that R^3 or R^4 represent a hydroxy, cyano or azido group or a halogen atom, wherein the residues may be the same or different.

8. Nucleoside monophosphate derivatives of formula I according to one of claims 1-7, characterized in that R^6 and R^7 represent a hydrogen atom; a C_1-C_6 alkyl residue optionally substituted by halogen; or a halogen atom; or a C_2-C_6 alkenyl or alkinyl group optionally substituted by halogen, wherein the residues may be the same or different.

9. Nucleoside monophosphate derivatives of formula I according to one of claims 1-8, characterized in

that R^8 represents a hydrogen atom, a C_1-C_6 alkyl residue, an amino group, or a halogen atom.

10. Nucleoside monophosphate derivatives of formula I according to one of claims 1-9, characterized in that R^{10} represents a hydrogen or halogen atom, a C_1-C_6 alkoxy, mercapto, C_1-C_6 alkylmercapto, or an amino group which may be mono- or disubstituted by a C_1-C_6 alkyl or a hydroxy-C_2-C_6 alkyl, or by a C_3-C_6 cycloalkyl, aryl or aralkyl residue, which optionally may further be substituted by one or more hydroxy, C_1-C_6 alkoxy or C_1-C_6 alkyl groups or by halogen atoms.

11. Nucleoside monophosphate derivatives of formula I according to one of claims 1-10, characterized in that

R^1 represents a straight-chain C_9-C_{13} alkyl group optionally further substituted by a methoxy, ethoxy, butoxy, hexyloxy, methylmercapto, ethylmercapto, propylmercapto, butylmercapto, or hexylmercapto residue;

R^2 represents a straight-chain C_8-C_{14} alkyl group optionally further substituted by a methoxy, ethoxy, propoxy, butoxy, hexyloxy, methylmercapto, ethylmercapto, butylmercapto, or hexylmercapto residue;

R^3 represents a hydroxy, cyano, azido or fluorine residue;

R^4 represents a hydroxy, cyano, azido or fluorine residue;

R^5 represents hydrogen;

R^6 represents hydrogen, a methyl, trifluoromethyl, ethyl, vinyl, propenyl, ethinyl, propinyl residue, or a fluorine, chlorine or bromine atom;

$R^{6'}$ represents hydrogen;

R^7 represents hydrogen, a methyl, ethyl, vinyl, propenyl, ethinyl, propinyl residue, or a chlorine or bromine atom;

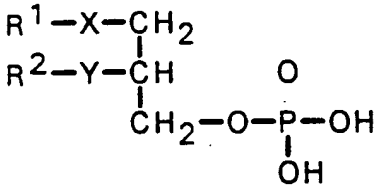
and/or

R^{10} represents hydrogen, a methoxy, ethoxy, propoxy, butoxy, hexyloxy, mercapto, methylmercapto, ethylmercapto, butylmercapto, hexylmercapto residue, an amino group optionally substituted by thienyl, furyl, pyridyl, thienylmethyl, furylmethyl, or pyridylmethyl.

12. Nucleoside monophosphate derivatives of formula I according to one of claims 1-11 characterized in that the nucleoside portion is selected from the following group: 6-mercaptopurine-9-ß-D-ribofuranoside, 5-fluorouridine, inosine, 5-methyluridine, 2',3'-didesoxy-2',3'-difluorothymidine, 5-chlorouridine, 5-trifluoromethyluridine, 5-ethinyluridine, 5-ethinylcytidine, 5-prop-1-enyluridine, 5-prop-2-enyluridine, adenosine, guanosine, 2,6-diaminopurine-9-ß-D-ribofuranoside, 2-amino-6-mercaptopurine-9-ß-D-ribofuranoside, 2-amino-6-methylmercaptopurine-9-ß-D-ribofuranoside, 2-amino-6-chloropurine-9-ß-D-ribofuranoside, 2'-desoxy-2'-aminoadenosine, 2'-desoxy-2'-azidoadenosine, 2'-desoxy-2'-azidocytidine, 2'-desoxy-5-fluorouridine, 2-chloroadenosine, 2-fluoroadenosine, 3'-desoxy-5-fluoroadenosine, 6-methylmercaptopurine-9-ß-D-ribofuranoside, 2-bromoadenosine, 2-fluoro-2'-desoxyadenosine.

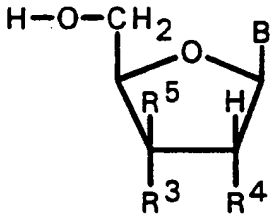
13. A process for the preparation of nucleoside monophosphate derivatives of formula I according to one of claims 1-12, characterized by

1. reacting a compound of general formula V



(V),

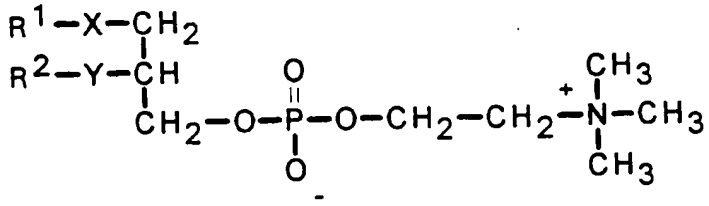
wherein R^1, R^2, X and Y have the meanings as indicated, with a compound of general formula VI



(VI),

wherein R^3, R^4, R^5 and B have the above mentioned meanings, or represent a hydroxy group protected by an oxygen protecting group familiar to the artisan, in the presence of an activating acid chloride, such as 2,4,6-triisopropylbenzenesulfonic acid chloride, and a tertiary nitrogen base, e.g., pyridine or lutidine, in an inert solvent, such as toluene, or immediately in anhydrous pyridine, and optionally, subsequent to hydrolysis, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry, or

2. reacting a compound of general formula VII



(VII),

wherein R^1, R^2, X and Y have the above-mentioned meanings, with a compound of general formula VI, wherein R^3, R^4, R^5 and B have the above-mentioned meanings, in the presence of phospholipase D from *Streptomyces* in an inert solvent such as chloroform, in the presence of a suitable buffer, and optionally, subsequent to reaction, removing the oxygen protecting groups according to procedures conventional in nucleoside chemistry.

14. A drug, containing at least one compound of formula I according to one of claims 1-12, as well as pharmaceutical adjuvants or vehicles.

15. Use of compounds of formula I according to one of claims 1-12 for the preparation of drugs for the treatment of tumors or human diseases.

Fig. 1

Influence of BM 92.0700 on the L1210 leukemia in vivo:

Survival time (Exp. 950001)

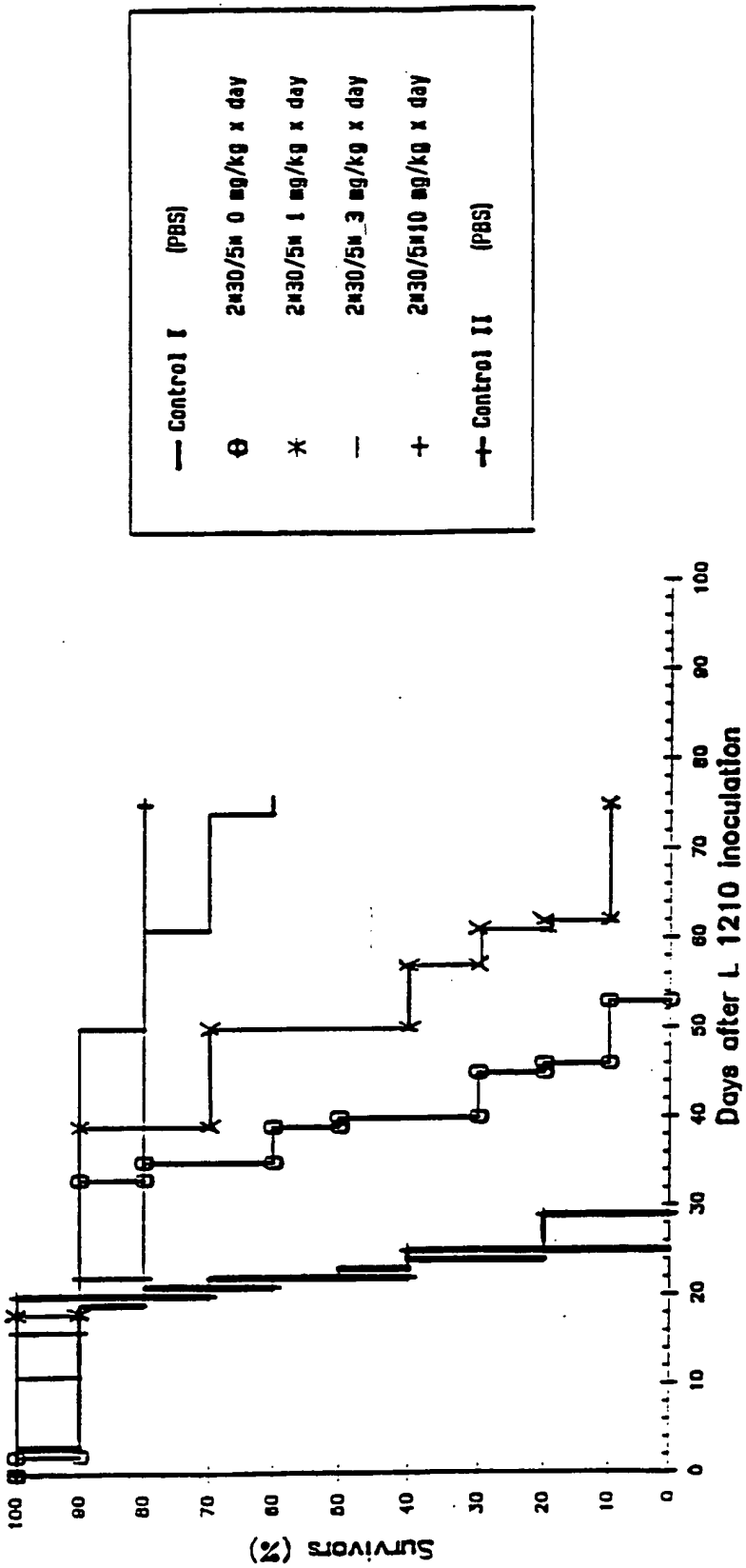
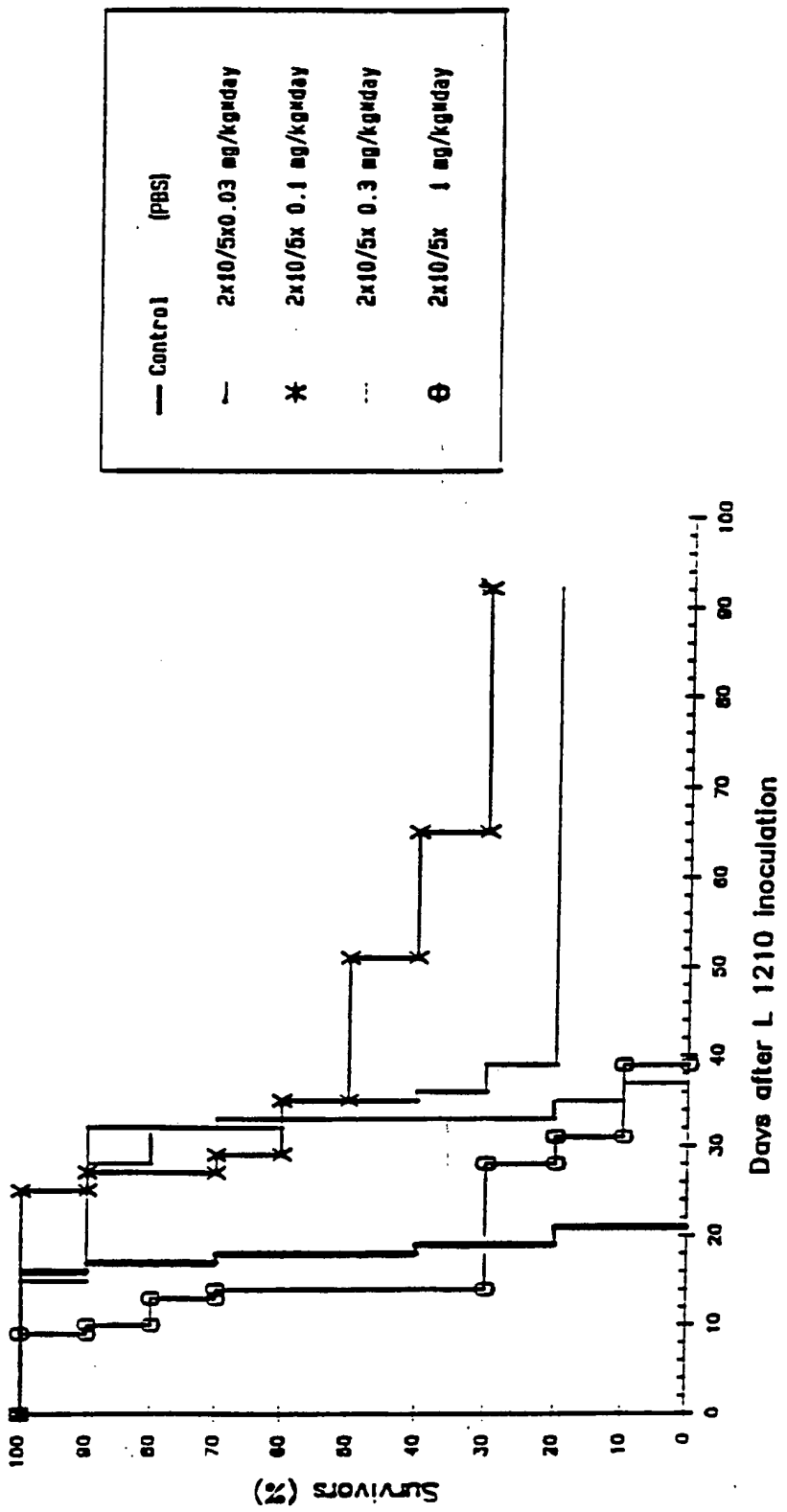


Fig. 2

Influence of 5-FU on the L1210 leukemia in vivo:

Survival time (Exp. 950066, Part II)



INTERNATIONAL SEARCH REPORT

Interna: I Application No
PCT/EP 95/01951

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-A-0 122 151 (MEITO SANGYO KK) 17 October 1984 see pages 8, 44, 53 and claim 1 ---	1-3,6-13
X,Y	EP-A-0 262 876 (TOYO JOZO KK) 6 April 1988 see the whole document ---	1-3,6-15
X	PATENT ABSTRACTS OF JAPAN vol. 012 no. 326 (C-525) ,5 September 1988 & JP,A,63 091090 (TOYO JOZO CO LTD) 21 April 1988, see abstract ---	1-3,6-13
Y	WO-A-93 16091 (BOEHRINGER MANNHEIM GMBH) 19 August 1993 see claim 1 ---	4,5
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 September 1995	10. 10. 95

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP-A-0 306 845 (HOECHST AG) 15 March 1989 see page 4, line 48 - line 53; claims 1-4	1-3, 6-12,14, 15
Y	DE-A-29 30 904 (GAURI KAILASH KUMAR DR) 19 February 1981 see claim 1	1-3, 6-12,14, 15
Y	WO-A-92 18520 (KNOLL AG) 29 October 1992 see the whole document	1-3,6-15
A	US-A-4 797 479 (SHUTO SATOSHI ET AL) 10 January 1989 see the whole document	1-15
Y	WO-A-92 03462 (BOEHRINGER MANNHEIM GMBH) 5 March 1992 cited in the application see claim 1	4,5

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Information on patent family members

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		JP-T- 6500543	20-01-94

1-(2,3,5-Tri-O-benzyl-β-L-arabinosyl)cytosine (15)

Cytosine 0.61 g (6 mmole) and ammonium sulfate (2 mg) were suspended in hexamethyldisilazane 15 ml, which was refluxed (140°C) under nitrogen for 2 hours to give a clear solution. The silylated cytosine mixture was evaporated to dryness *in vacuo* while avoiding of contact with moisture to give a syrup 14.

Compound 9 (2.82 g, 5 mmole) was added to 47 ml of dry dichloromethane presaturated with anhydrous hydrogen chloride at 0°C. After 2 hours at 0°C, the precipitated p-nitrobenzoic acid (0.807 g) was removed by filtration, and the filtrate was concentrated *in vacuo* to give syrupy 2,3,5-tri-O-benzyl-α-L-arabinosyl chloride (10).

Compound 10 thus prepared was dissolved in 28.5 ml of anhydrous dichloromethane, and the solution was added to a mixture of silylated cytosine (14) and 8.3 g of 4A molecular sieve. The reaction mixture was stirred at room temperature under nitrogen for 5 days. Then the reaction mixture was then diluted with 50 ml of dichloromethane and 20 ml of water, and poured into 2 ml of saturated aqueous $NaHCO_3$ under vigorous stirring. White precipitate (tin hydroxide) appeared, which was filtered out on the bed of celite. The organic layer was separated from water and washed with water (3 X 30 ml). The aqueous layer was extracted with dichloromethane, and the combined dichloromethane layer was dried over Na_2SO_4, and then evaporated *in vacuo* to give a syrup which was purified by silica gel column chromatography (chloroform: methanol, 96:4) to give 15 as a white solid which was recrystallized with 2-propanol to give 1.53 g (60%). m.p. 146-148°C, $[\alpha]^{25}_D$=-105.2 (Cl, CH_2Cl_2), UV(MeOH): λ_{max} 211.5, λmin 272.5, pH1,

pH7; λmax 211.5, λ_{min} 284, pH9. 300MHz 1HNMR($CDCl_3$) δ: 3.65 (d, 2H, J=4.85, H-5'), 4.00 (t, 1H, J=3.72, H-3'), 4.11 (m, 1H, H-4'), 4.28 (m, 1H, H-2'), 4.38-4.55 (m, 6H, benzyl CH_2), 5.56 (d, 1H, J-7.5, H-5), 6.39 (d, 1H, J=4.59, H-1'), 7.12-7.31 (m, 15H, H-aromat), 7.63 (d, 1H, J=7.5, H-6).

1-β-L-Arabinofuranosylcytosine Hydrochloride Salt(16) by Catalytic Hydrogenation of 15

Palladium chloride (315 mg, 1.78 mmole) was suspended in 160 ml of methanol, and reduced by shaking with hydrogen at room temperature. To the acidic suspension of palladium black was then added a solution of 300 mg of 15 in 54 ml of methanol. The reaction mixture was shaken with hydrogen at room temperature for 3 hours. After the catalyst had been removed, the solution was neutralized with Dowex (HCO_3^-), concentrated *in vacuo* and then purified by preparative TLC (MeOH:$CHCl_3$, 3:5) to give a syrup which was dissolved in 3 ml of methanol, added 1% HCl solution in MeOH to pH1, concentrated to dryness and triturated with 2-propanol to give 36 mg (22.1%) of 16. m.p. 190-194°C, $[\alpha]^{25}_D$=-115.47 (C 0.07, H2O); UV(H2O) λ_{max} 275, pH7; λ_{max} 209.5, 273, pH11; λ_{max} 280, pH1; 300 MHz 1HNMR(DMSO-d6); δ 3.61 (d, 2H, H-5'), 3.82 (m, 1H, H-4'), 3.93 (m, 1H, H-2' or H-3'), 4.04 (br s, 1H, H-2') or H-3'), 5.18 (br s, 1H, C5'-OH, exchangeable), 5.61 (br s, 1H, C2'-OH or C3'-OH, exchangeable), 5.67 (br s, 1H, C2'-OH or C3'-OH, exchangeable), 6.00 (d, 1H, J=4.02, H-1'), 6.01 (d, 1H, $J_{5,6}$=7.8, H-5) 7.92 (d, 1H, $J_{5,6}$=7.8, H-6), 8.49 (br s, 1H, NH, exchangeable), 9.38 (br s, 1H, NH, exchangeable).

1-β-L-Arabinofuranosylcytosine Hydrochloride Salt (16) by Treatment of Compound 15 with Boron Trichloride

5 ml of 1M boron trichloride in dichloromethane was cooled at -72°C (dry ice-acetone). A solution of 15 (180 mg, 0.351 mmole) in 3 ml of dichloromethane was added slowly to the boron trichloride solution. After a total reaction time of 2.75 hours, the cooling bath was removed and the solvent and gas were removed *in vacuo*. The residue was dissolved in cold dichloromethane (10 ml) and the solution was evaporated to dryness (3 times, until a white solid residue was obtained), cold saturated sodium hydrogen carbonate solution was added to adjust the pH to 6-7. The mixture was diluted with ethanol, heated to boiling, treated with charcoal, and filtered. The filtrate was evaporated to dryness to a syrup which was dissolved 3 ml of methanol, added 1% HCl solution in methanol to pH 1, concentrated to dryness and triturated with 2-propanol to give 16 (66 mg, 78.4%).

9-(2,3,5-Tri-O-benzyl-β-L-arabinosyl) adenine (18)

Thoroughly dried compound 9 (5 g, 8.8 mmole) was added to 82 ml of dichloromethane presaturated with anhydrous hydrogen chloride at 0°. After 2 hours of reaction at 0°, the precipitated p-nitrobenzoic acid (1.53 g) was removed by filtration, and the filtrate was concentrated *in vacuo* to a syrup which was then kept in full vacuo at room temperature for 2 hrs. The 2,3,5-tri-O-benzyl-α-L-arabinosyl chloride (10) thus prepared was dissolved in 50 ml of dichloromethane, and the solution was added to a mixture of 4.5 g (18.8 mmole) of dried N-benzoyladenine[5] (17) and 14.5g of 4A molecular sieve. The reaction mixture was stirred at room

temperature for 1 week, filtered through a bed of celite, and concentrated *in vacuo* to a syrup which was chromatographed on silica gel using hexanes-acetone (3:1, Rf=0.22). The product was separated and concentrated *in vacuo* to a syrup which was dissolved and stirred with methanolic ammonia (20 ml) in a stainless-steel bomb, and heated overnight at 50-55°C. The solution was then concentrated at reduced pressure to give a semisolid which was recrystallized from warm isopropyl alcohol to give 18 2.4g (50.7%). m.p. 128-129°C. $[\alpha]^{27}_{D}$=-20.04 (1.04, CH_2Cl_2); UV(CH_2Cl_2): λ_{max} 213, 258.5; 250MHz 1HNMR($CDCl_3$): δ 1.95 (br s, 1H, NH, exchangeable), 3.69 (d, 2H, J=4.82, H-5'), 4.18-4.30 (m, 6H, benzyl CH_2), 4.51-4.64 (m, 3H, H-2',3',4'), 5.73 (br s, 1H, NH, exchangeable), 6.52 (d, 1H, J-4.00, H-1'), 6.89-6.93, 7.17-7.37 (m, 15H, H-aromat of benzyl group), 8.17 (s, 1H, H-2 or H-8), 8.32 (s, 1H, H-2 or H-8).

9-β-L-Arabinofuranosyladenine (19)

Boron trichloride (5 ml of 1M) in dichloromethane was cooled at -72°C (Dry Ice-acetone). A solution of 18 (150 mg, 0.279 mmole) in 5ml of dichloromethane was added slowly to the boron trichloride solution. After a total reaction time of 3.5 h, the cooling bath was removed, and the solvent and gas were removed *in vacuo*. The residue was dissolved in cold dichloromethane (10 ml) and the solution was evaporated to dryness (6 times, until a yellow solid residue was obtained). Cold saturated sodium hydrogen carbonate solution was added to adjust the pH 7-8. The mixture was diluted with ethanol, heated to boiling, the suspension filtered through celite, the filtrate was concentrated *in vacuo* to a syrup which was crystallized with water. 55 mg (74%) of 19 was

obtained. m.p. 256-258°C, UV (H_2O): λ_{max} 259; 300 MHz 1HNMR(DMSO-d6): δ: 3.62-3.66 (m, 2H, H-5'), 3.77 (br s, 1H, H-4'), 4.13 (br s, 2H, H-2', 3'), 5.12 (t, 1H, J=5.4, C'5-OH, exchangeable), 5.54 (d, 1H, J=3.78, C'2-OH or C'3-OH, exchangeable), 5.63 (d, 1H, J=4.32, C'2-OH or C'3-OH, exchangeable), 6.25 (d, 1H, J=4.02, H-1'), 7.25 (br s, 2H, NH2, exchangeable), 8.13 (s, 1H-H-2 or H-8), 8.18 (s, 1H, H-2 or H-8).

Figure 10 is an illustration of an alternative route for the preparation of 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-L-ribofuranose (compound 10) from 1,2-di-O-isopropylidene-α-L-xylofuranose (compound 3).

1,2-Di-O-isopropylidene-α-L-xylofuranose (3)

To 650 ml of anhydrous acetone was added 4 ml of conc. sulfuric acid, 5 g of molecular sieve (4A), 80 g of anhydrous cupric sulfate and 40 g of L-xylose. The mixture was stirred at r.t. for 36 hrs. The reaction mixture was then filtered and washed thoroughly with acetone, the combined filtrate was neutralized with ammonium hydroxide then evaporated to dryness. Ethyl acetate (200 ml) was added, and then filtered and evaporated, yielding an oil which was dissolved in 250 ml of 0.2% HCl and solution and stirred at room temperature for 2.5 hrs. The pH was adjusted to 8 with sat. $NaHCO_3$, and then evaporated to dryness. The residue was extracted with ethyl acetate. Removal of the solvent provided a yellow oil of 3 (41.7g, 82.3%). ^{1}H-NMR($CDCl_3$): δ 5.979 (d, J=3.78Hz,1H, H-1); 4.519 (d, J=3.6Hz, 1H, H-2); 4.308 (bd, 1H, H-3); 4.080 (m, 3H, H-4 and H-5); 1.321 (s, 3H, CH_3); 1.253 (s, 3H, CH_3).

5-O-Benzoyl-1,2-di-O-isopropylidene-α-L-xylofuranose (25)

Compound 3 (41 g, 215.6 mmol) was stirred in pyridine (150 ml) and CH_2Cl_2 (150 ml) at 0 °C. BzCl (27.5 ml, 237 mmol) dissolved in 30 ml of pyridine was added dropwise. After 30 min, water (5 ml) was added and the mixture was evaporated to dryness, dissolved in EtOAc, washed with sat. $NaHCO_3$, and then dried (Na_2SO_4). Evaporation of solvent gave an orange syrup which was crystallized in Et_2O to give compound 20 (36 g). The mother liquor was evaporated to dryness, and the residue purified by silica gel column chromatography (1% $CH_3OH/CHCl_3$) to give another batch of compound 25 (12 g, total yield 76%). m.p. 82-83°C. lit[1] D form: 83.5-84.5 °C. ^{1}H-NMR ($CDCl_3$): δ 7.43, 8.07 (m, 5H, Ar-H); 5.97 (d, 1H, J=3.6Hz, H-1); 4.80 (q, 1H, H-4); 4.60 (d, 1H, J=3.6Hz, H-2); 4.40, 4.20 (m, 3H, H-5, H-5', H-3); 3.50 (bs, 1H, D_2O exchange, 3OH); 1.51, 1.32 (2s, 6H, $2CH_3$).

5-O-Benzoyl-1,2-di-O-isopropylidene-α-L-erythro-pentofuranos-3-ulose (26)

Compound 25 (40 g, 136 mmol) was stirred in 450 ml of CH_2Cl_2. To this was added pyridinium dichromate (PDC, 30.7 g, 81.6 mmol) and Ac_2O (42.3 ml, 448.8 mmol). The mixture was refluxed for 2.5 hrs. The solution was concentrated to 1/5 its original volume, and then ethyl acetate (50 ml) was added, and the solution filtered. The filtrate was poured onto a silica gel pad (10 cm x 5 cm), eluted with EtOAc, the combined eluant was concentrated, and coevaporated with toluene (50 ml x 2). Cyrstallization from hexane and EtOAc gave **21** as a white solid (38 g, 96%). m.p 91-93°C, $[\alpha]_D$: -132° (c,1.0, $CHCl_3$); lit[2] D form: m.p. 93-94.5°C, $[\alpha]_D$: +135° (c,1.0, $CHCl_3$); IR (KBr): 1773cm- (ArCO), 173°Cm- (CO). ^{1}H-NMR ($CDCl_3$): δ 7.97, 7.42 (m, 5H,

Ar-H); 6.14 (d, 1H, J=4.4Hz, H-1); 4.74, 4.68 (m, 2H, H-4, H-2); 4.50 4.44 (m, 2H, H-5, H-5'); 1.52, 1.44 (2s, 6H, $2CH_3$). Anal. Calcd. ($C_{15}H_{16}O_6$): C, 61.64; H, 5.52; Found: C, 61.42; H, 5.53.

5-O-Benzoyl-1,2-di-O-isopropylidene-α-L-erythro-pentofuranos-3-ulose (26)

Compound 25 (40 g, 136 mmol) was stirred in 450 ml of CH_2Cl_2. To this was added pyridinium dichromate (PDC, 30.7 g, 81.6 mmol) and Ac_2O (42.3 ml, 448.8 mmol). The mixture was refluxed for 2.5 hrs. The solution was concentrated to 1/5 its original volume, and then EtOAc (50 ml) was added. The solution was filtered, the filtrate was poured onto a silica gel pad (10 cm x 5 cm), eluted with EtOAc, the combined eluant was concentrated, and coevaporated with toluene (50 ml x 2). Cyrstallization from hexane and EtOAc gave **26** as a white solid (38 g, 96%). m.p 91-93°C, $[\alpha]_D$: -132° (c,1.0, $CHCl_3$); lit[2] D form: m.p. 93-94.5°C, $[\alpha]_D$: +135° (c,1.0, $CHCl_3$); IR (KBr): 1773cm- (ArCO), 173°Cm- (CO). ^{1}H-NMR ($CDCl_3$): δ 7.97, 7.42 (m, 5H, Ar-H); 6.14 (d, 1H, J=4.4Hz, H-1); 4.74, 4.68 (m, 2H, H-4, H-2); 4.50 4.44 (m, 2H, H-5, H-5'); 1.52, 1.44 (2s, 6H, $2CH_3$). Anal. Calcd. ($C_{15}H_{16}O_6$): C, 61.64; H, 5.52; Found: C, 61.42; H, 5.53.

1,2-Di-O-isopropylidene-α-L-ribofuranose (27)

Compound **26** (37 g, 127 mmol) was dissolved in EtOH/H_2O (400 ml/100 ml) at 0°C, while $NaBH_4$ (23.3 g, 612 mmol) was added portionwise. The suspension was stirred at r.t. for 4 hrs. It was filtered and the filtrate evaporated to dryness and coevaproated with methanol. After silica gel column chromatography (0-15% CH_3OH/CH_2Cl_2) and crystallization from EtOAc/Hexane, **27** was obtained as white needle (19 g, 79%). m.p. 86-87°C; $[\alpha]_D$-31.5° (c, 0.62, $CHCl_3$); lit[3], D form: m.p. 86-87°C, $[\alpha]_D$:+37° (c, 0.59, $CHCl_3$); IR (KBr): 3356 cm-

(OH). ^{1}H-NMR ($CDCl_3$) δ 5.83 (d, 1H, J=3.98Hz, H-1); 4.595 (t, 1H, H-2); 4.055, 3.72 (m, 4H, H-3, H-4, H-5, H-5'); 2.38 (d, 1H, D_2O exchange, 3-OH); 1.83 (t, 1H, D_2O exchange, 5-OH); 1.58, 1.38 (2s, 6H, $2CH_3$). Anal. Calcd. ($C_8H_{14}O_5$): C, 50.50; H, 7.42; Found: C, 50.62; H, 7.46.

3,5-Di-O-Benzoyl-1,2-di-O-Isopropylidene-α-L-ribofuranose (28)

Compound **27** (19 g, 100 mmol) was stirred in 300 ml of pyridine at 0°C, while BzCl (40 ml, 348 mmol) was added dropwise and then stirred at 4.t. for 3 hrs. The solvent was evaporated to dryness. The residue was extracted with EtOAc, washed with sat. $NaHCO_3$, dried (Na_2SO_4), evaporation of solvent and crystallization in either gave **23** as a white solid of 39g (98%) m.p. 83-85°C. ^{1}H-NMR ($CDCl_3$): δ 8.07, 7.36 (m, 10H, Ar-H); 5.94 (d, 1H, J=3.6Hz, H-1); 5.05, 5.00 (m, 1H, H-2); 4.73, 4.63 (m, 3H, H-4, H-5, H-5'); 1.58, 1.35 (2s, 6H, $2CH_3$). Anal. Calcd. ($C_{22}H_{22}O_7$): C, 66.50; H, 5.64; Found: C, 66.32; H, 5.57.

1-O-Acetyl-2,3,5-tri-O-benzoyl-ß-L-ribofuranose (31)

Compound **28** (38 g, 95 mmol) was stirred in 300 ml of 1% HCl/CH_3OH at r.t. for 30 hrs. Pyridine (20 ml) was added and then the solution was evaporated to dryness. The residue was coevaporated with pyridine (30 ml x 2), and then dissolved in 100 ml of anhydrous pyridine at 0°C while BzCl (17 ml, 146 mmol) was added dropwise, then stirred at r.t. for 3 hrs. The solvent was evaporated, and the residue was dissolved in EtOAc, washed with 0.5 N HCl and then sat. $NaHCO_3$, and then dried (Na_2SO_4). Evaporation of solvent gave crude **30** as a syrup.

The crude **30** was stirred in glacial acetic acid (400 ml), and then Ac_2O (100 ml) at 0°C while conc. H_2SO_4 (10 ml) was added dropwise. This solution was

stirred at r.t. overnight. The mixture was poured into ice-water, extracted with $CHCl_3$, neutralized with sat. $NaHCO_3$, and then dried (Na_2SO_4). After evaporation of solvent, a light yellow syrup was obtained, which was crystallized in methanol to give **31** as a white solid of 23.89 g (49.6%, from **28-31**). m.p.124.7 °C, $[\alpha]_D$=45.613 (c 1.0, $CHCl_3$); lit[4], m.p.:129-130 °C, $[\alpha]_D$=-43.6 (c 1.0, $CHCl_3$). ^{1}H-NMR($CDCl_3$): δ 7.317, 8.134 (m, 15H, OBz); 6.437 (s, 1H, H-1); 5.835 (m, 2H, H-2 and H-3); 4.649 (m, 3H, H-4 and H-5); 2.003(s, 3H, $\underline{CH_3}COO$-)

II. Biological Activity

The compounds disclosed herein can be evaluated for activity against HBV and EBV as disclosed in detail below, or by other methods known to those skilled in the art.

Example 1 Biological Activity Against HBV

Human hepatoma cells with HBV (2.2.15 cells) were used in the assay. These cells were grown to confluence in minimal essential medium with 10% fetal bovine serum. The media was changed at 3-day intervals. At confluence the treatment with the drugs was initiated and then continued for three days. Another addition of the same concentration of the drug was given at this point after removal of the media from the cultures and cultures maintained for an additional period of 3 days. The medium after the 6-day treatment was harvested and the viral particles precipitated by the polyethylene glycol method. The particles were subsequently digested and then processed for Southern analysis. The blots were hybridized to an HBV-specific probe and viral DNA amounts assessed in comparison to DNA levels from cultures not

treated with the drugs. The genomic DNA was digested with Hind III and subjected to Southern analysis. Levels of the episomal DNA were determined in relation to the integrated HBV DNA. The drug concentrations that cause 50% inhibition of the DNA (ID_{50}) as compared to the controls were calculated. The results are summarized in Table I.

Table 1

Inhibition of HBV by L-Nucleosides

Compound	Anti-HBV Activity ID_{50} (μM)	MT2 CD_{50} (μM)	CEM CD_{50}	CD_{50} (μM) (H1 cells)
L-FMAU	0.1	100	>100	1000
D-FMAU	2.0	8-9	10	50
D-FEAU	5.0	100	90	2000
L-FEAU	>5.0	100	>100	900

Example 2 Biological Activity Against EBV

H1 cells were maintained in long phase growth for two days prior to the initiation of treatment. The cells were centrifuged at 600 g for 10 min. at room temperature to separate them from the medium containing pre-existing virus particles. The H1 cells were seeded in 24 well plates at a density of 1×10^5 cells per well in 2 ml of fresh medium with or without drug and incubated at 37°C for 5 days. The medium containing virion was saved and used for evaluating the inhibitory effect of drugs on virus production and infectivity by use of a bioassay. The virion were pelleted from the cell free medium by centrifugation at 45,000 rpm for 90 min. in a SW-50 Ti rotor (Beckman). The virion were resuspended in 1 ml growth medium and then used to infect 1×10^6 Raji cells for 48 hours. Since the level of EBV DP activity in Raji cells post superinfection is proportional to the number of virions added, the EBV specific DP activity induced was able to be measured. The inhibitory drug effect was calculated by comparing the EBV DP activity to multiple dilution controls. No inhibition of mitochondrial DNA content in H1 cells was observed when the cells were treated by 1 mM L-FMAU for 6 days.

Slot Blot Assay - The amount of mitochondrial DNA was measured by the slot blot method (2). 2×10^5 of the treated and non-treated H1 cells were lysed in 200 µl of 10 mM of Tris.HCl (pH 7.5) solution by the freeze/thaw method. The cell lysate was treated with 10 µg/ml of RNase A at 37°C for 30 min, and then proteinase K (100 µg/ml) at 55°C for 2 hours. Equal amounts of 20 X SSC buffer was added to each cell lysate. After boiling for 10 min, the samples were spotted onto nylon membranes (Hybond-N, Amersham Corp.). A

radiolabelled human mitochondrial DNA fragment was used as a probe for DNA hybridization. The same membranes were reprobed with human Alu DNA after removing the mitochondrial DNA probe. The amount of mitochondrial DNA in the treated and non-treated H1 cells was quantified by densitometer (LKB Ultroscan XL).

The results are provided in Table 2.

Table 2

Drugs	CD_{50} (μM)	ID_{90} (μM)	Therapeutic Index (CD_{50}/ID_{90})
PFA	1200 ± 100	75 ± 5	16
DHPG	75 ± 6	5 ± 1	15
ACV	1000 ± 75	50 ± 8	20
PCV	500 ± 55	20 ± 2	25
L-FMAU	1000 ± 80	5 ± 0.8	200
D-FMAU	50 ± 10	0.1 ± 0.02	50
L-FEAU	900 ± 70	60 ± 11	15
D-FEAU	2000 ± 80	1 ± 0.05	2000
L-FIAC	400 ± 35	< 10	> 40
D-FIAC	125 ± 15	5 ± 0.5	25
L-FIAU	240 ± 20	20 ± 4	12
D-FIAU	20 ± 4	0.5 ± 0.05	40

Inhibitory effect of compounds on EBV: The CD_{50} was performed by exposing H1 cells to different concentrations of the compounds in normal growth medium at 37°C for 72 hours; the cells were then counted and compared with controls. H1 cells were treated for 5 days and the ID_{90} was determined by bioassay.

Example 3 Clearance of L-(-)-FMAU from Plasma and Liver

The clearance of L-(-)-FMAU from the plasma and liver of mice after oral administration was evaluated. Mice were injected with L-(-)-FMAU which was tritium labeled (radiospecificity of 9.3 μmol/μCi).

Figure 11 is a graph of the plasma concentration of L-(-)-FMAU in mice after oral administration over time, and Figure 12 is a graph of the concentration of L-(-)-FMAU in mouse liver after oral administration over time (cross, 10 mg/kg administered bid (bi-daily) for 30 days prior to pharmacokinetic study and then study carried out on day thirty one on administration of same concentration; dark circle, 50 mg/kg administered bid for 30 days prior to study and then study carried out on day thirty one on administration of same concentration; open circle, 50 mg/kg administered for the first time on the first day of the study).

At the times indicated (see Figures 10 and 11) the mice were bled from their retro-orbital sinus using heparinized capillaries. The plasma was extracted with trichloroacetic acid and neutralized with freon/trioctylamine. Supernatants were directly counted and the concentration calculated.

As indicated in Figures 10 and 11, the peak concentration of L-(-)-FMAU in the plasma and liver occurred at approximately one hour. The compound was substantially cleared from both plasma and liver after four hours.

Example 4 Toxicity of L-(-)-FMAU in BDF1 Female Mice

Figure 13a illustrates the change in body weight over thirty days of control BDF1 female mice.

Figures 13b and 13c illustrate the change in body weight over thirty days of BDF1 female mice dosed with 10 mg/kg (13b) and 50 mg/kg (13c) bid of L-(-)-FMAU. The body weight presented represents the mean and standard deviation of that of five to seven mice. As indicated in Figures 13b and 13c, L-(-)-FMAU did not appear to significantly affect the weight of the mice over thirty days, indicating that the compound was well tolerated.

Example 5 Clinical Chemistry of Mouse Plasma after L-(-)-FMAU Treatment

Figures 14-20 provide the clinical chemistry of mouse plasma after administration of L-(-)-FMAU at 10 mg/kg (three mice) or 50 mg/kg (three mice) bid for thirty days.

Figure 14 is a bar chart graph of the concentration of total bilirubin in the mouse plasma in mg/dL. Figure 15 is a bar chart graph of the concentration of alkaline phosphatase in the mouse plasma in U/L. Total bilirubin and alkaline phosphatase are indicators of liver function. The mouse values for the bilirubin fall within the normal human range (less than 1.2 mg/dL), but those for alkaline phosphatase are somewhat higher than the normal human level (30-114 U/L).

Figure 16 is a bar chart graph of the concentration of creatinine in the mouse plasma in mg/dL. Creatinine is an index of kidney function. With the exception of mouse 50-2, the creatinine levels of the treated mice are not different from those of the control mice.

Figure 17 is a bar chart graph of the concentration of AST (SGOT, serum glutamic oxalic transaminase) in the mouse plasma in U/L. Figure 18 is a bar chart graph of the concentration of ALT (SGPT, serum glutamic pyruvic transaminase) in the

mouse plasma in U/L. SGOT and SGPT are indicators of liver function. With the exception of mouse 50-2 (for both SGOT and SGPT) and mouse 10-2 (for SGOT only), the enzyme levels of the treated mice are not different from those of the control mice.

Figure 19 is a bar chart graph of the concentration of lactic acid in the mouse plasma in mmol/L. Figure 20 is a bar chart graph of the concentration of lactic dehydrogenase in the mouse plasma in U/L. Lactic acid is formed in muscles during glycolysis. Lactic dehydrogenase (LDH) is present as different isoenzymes in different tissues. Release of LDH into plasma may be an indication of tissue damage. The lactic acid and lactic dehydrogenase levels of the treated mice are not significantly different from those of the control mice.

III. Oligonucleotides

Oligonucleotides of desired sequences can be modified by substitution of one or more of the L-nucleosides disclosed herein for a nucleoside in the oligonucleotide. In a preferred embodiment the L-nucleoside is placed at one of the termini of the oligonucleotide. The modified oligonucleotide can be used, for example, in antisense technology.

Antisense technology refers in general to the modulation of gene expression through a process wherein a synthetic oligonucleotide is hybridized to a complementary nucleic acid sequence to inhibit transcription or replication (if the target sequence is DNA), inhibit translation (if the target sequence is RNA) or to inhibit processing (if the target sequence is pre-RNA). A wide variety of cellular activities can be modulated using this technique. A simple example is the inhibition of protein biosynthesis by an antisense

oligonucleotide bound to mRNA. In another embodiment, a synthetic oligonucleotide is hybridized to a specific gene sequence in double stranded DNA, forming a triple stranded complex (triplex) that inhibits the expression of that gene sequence. Antisense oligonucleotides can be also used to activate gene expression indirectly by suppressing the biosynthesis of a natural repressor. AOT can be used to inhibit the expression of pathogenic genes, for example, those that facilitate the replication of viruses, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and herpesviruses, and cancers, particularly solid tumor masses such as gliomas, breast cancer, and melanomas.

The stability of a selected oligonucleotide against nucleases is an important factor for in vivo applications. It is known that 3'-exonuclease activity is responsible for most of the unmodified antisense oligonucleotide degradation in serum. Vlassov, V.V., Yakubov, L.A., in Prospects for Antisense Nucleic Acid Therapy of Cancers and AIDS, 1991, 243-266, Wiley-Liss, Inc., New York; Nucleic Acids Res., 1993, 21, 145. In one embodiment, the L-nucleosides disclosed herein can be used to minimize 3'-exonuclease degradation of antisense oligonucleotides.

Oligonucleotides of the present invention which are capable of binding to polyribonucleic acid or polydeoxyribonucleic acid are useful as antisense agents in the same manner as conventional antisense agents. See generally Antisense Molecular Biology and S-oligos, *Synthesis* 1 (Oct. 1988) (published by Synthecell Corp., Rockville, Md.); 2 Discoveries in Antisense Nucleic Acids (C. Brakel and R. Fraley eds. 1989); Uhlmann, et. al., "Antisense Oligonucleotides: A New Therapeutic Technique,"

Chem. Rev. **90(4)**, 1990; and Milligan, J.F., Matteucci, M.D., Martin, J.C., J. Med. Chem., 1993, 36, 1923-1937. Antisense agents of the present invention may be used by constructing an antisense agent which is capable of selectively binding to a predetermined polydeoxyribonucleic acid sequence or polyribonucleic acid sequence to a cell containing such sequence (e.g., by adding the antisense agent to a culture medium containing the cell) so that the antisense agent is taken into the cell, binds to the predetermined sequence, and blocks transcription, translation, or replication thereof. The requirements for selective binding of the antisense agent are known (e.g., a length of 17 bases for selective binding within the human genome).

IV. Preparation of Pharmaceutical Compositions

The compounds disclosed herein and their pharmaceutically acceptable salts, prodrugs, and derivatives, are useful in the prevention and treatment of HBV and EBV infections and other related conditions such as anti-HBV or anti-EBV antibody positive and HBV- or EBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV anti-EBV antibody or HBV- or EBV-antigen positive or who have been exposed to HBV or EBV.

Humans suffering from any of these conditions can be treated by administering to the patient an effective HBV- or EBV- treatment amount of one or a

mixture of the active compounds described herein or a pharmaceutically acceptable derivative or salt thereof, optionally in a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount without causing serious toxic effects in the patient treated.

A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art. In one embodiment, the active compound is administered as described in the product insert or Physician's Desk Reference for 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), or 2',3'-dideoxy-2',3'-didehydrothymidine (D4T) for HIV indication.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage

form. A oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The active compound can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of the nucleosides that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with cations such as sodium, potassium, zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylene-diamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N^6 or N^4 and 5'-O positions, can

affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a

disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV, anti-EBV, anti-cytomegalovirus, or anti-HIV or anti-EBV agents.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as

ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS). In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

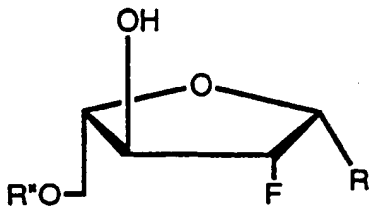
Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the

surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim.

1. An L-nucleoside compound of the formula:

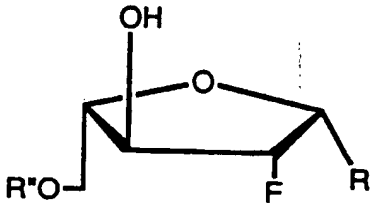


wherein R is a purine or pyrimidine base and R" is hydrogen, acyl, alkyl, monophosphate, diphosphate, or triphosphate.

2. Use of a compound of claim 1 and pharmaceutically acceptable salts thereof in the manufacture of a medicament for treatment of an EBV or HBV infection.

3. An L-nucleoside of claim 1 and pharmaceutically acceptable salts thereof in the manufacture of a medicament for the treatment of an EBV or HBV infection.

4. A method for the treatment of a human infected with HBV or EBV is provided that includes administering an HBV- or EBV-treatment amount of an L-nucleoside of the formula:



wherein R is a purine or pyrimidine base and R" is hydrogen, acyl, alkyl, monophosphate, diphosphate, or triphosphate.

5. The method of claim 4, wherein the L-nucleoside is 2'-fluoro-5-methyl-ß-L-arabinofuranosyluridine.

6. The L-nucleoside of claim 1 that is 2'-fluoro-5-methyl-ß-L-arabinofuranosyluridine.

7. The L-nucleoside of claim 1 or 3 selected from the group consisting of N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-ethyluracil, N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodocytosine), and N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodouracil.

8. The L-nucleoside of claim 1 or 3, wherein the base is selected from the group consisting of 5-methyl uracil (thymine), 5-iodouracil, cytosine, and 5-ethyluracil.

9. The use of claim 2, wherein the L-nucleoside is 2'-fluoro-5-methyl-ß-L-arabinofuranosyluridine.

10. The use of claim 2, wherein the L-nucleoside is selected from the group consisting of N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-ethyluracil, N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodocytosine), and N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodouracil.

11. The use of claim 2, wherein the base is selected from the group consisting of 5-methyl uracil (thymine), 5-iodouracil, cytosine, and 5-ethyluracil.

12. The method of claim 4, wherein the L-nucleoside is selected from the group consisting of N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-ethyluracil, N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodocytosine), and N_1-(2'-deoxy-2'-fluoro-ß-L-arabinofuranosyl)-5-iodouracil.

13. The method of claim 4, wherein the base is selected from the group consisting of 5-methyl uracil (thymine), 5-iodouracil, cytosine, and 5-ethyluracil.

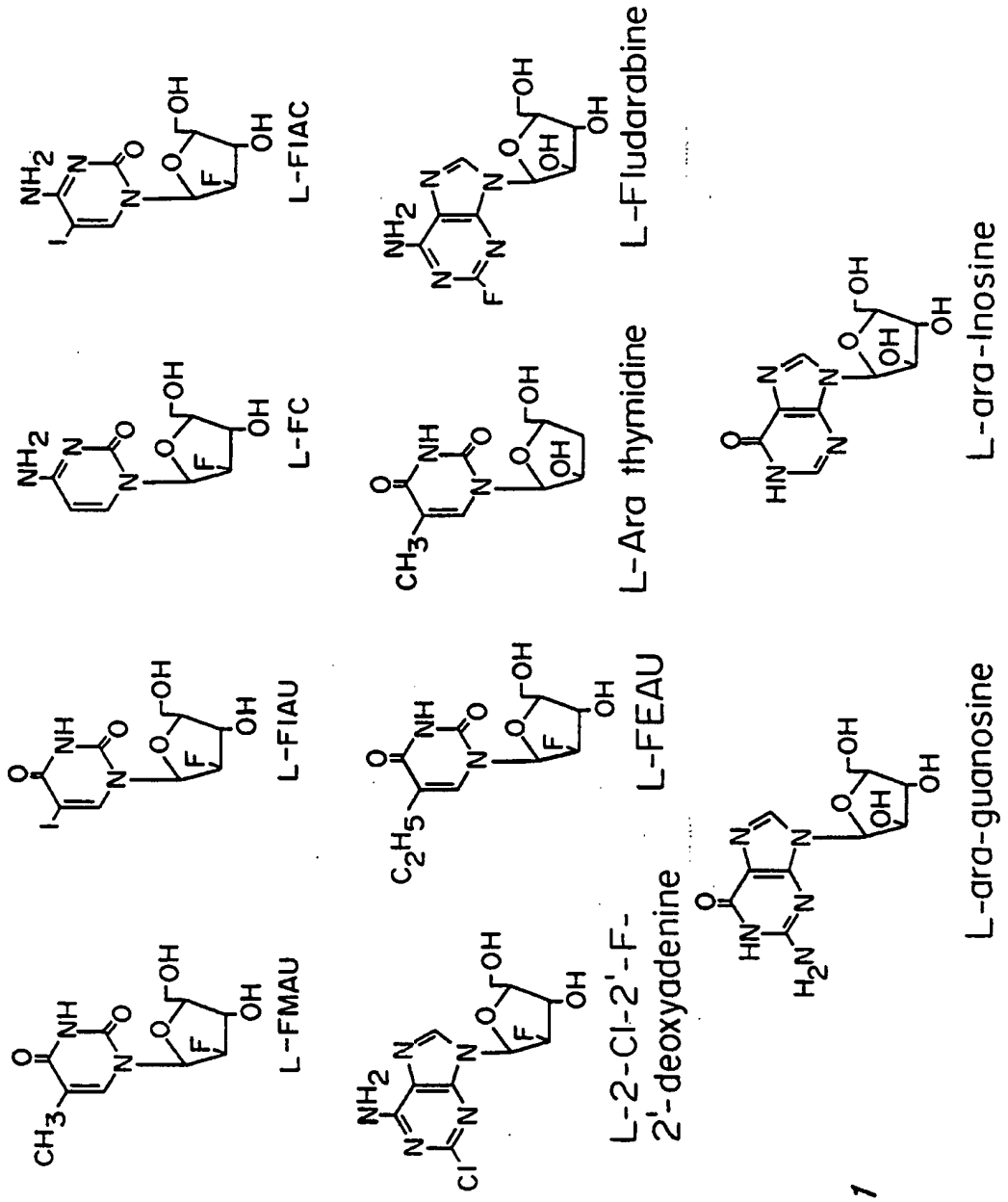

L-FIAC
L-FC
L-Fludarabine
L-Ara thymidine
L-FIAU
L-FEAU
L-ara-Inosine
L-FMAU
L-2-Cl-2'-F-
2'-deoxyadenine
L-ara-guanosine

FIG. 1

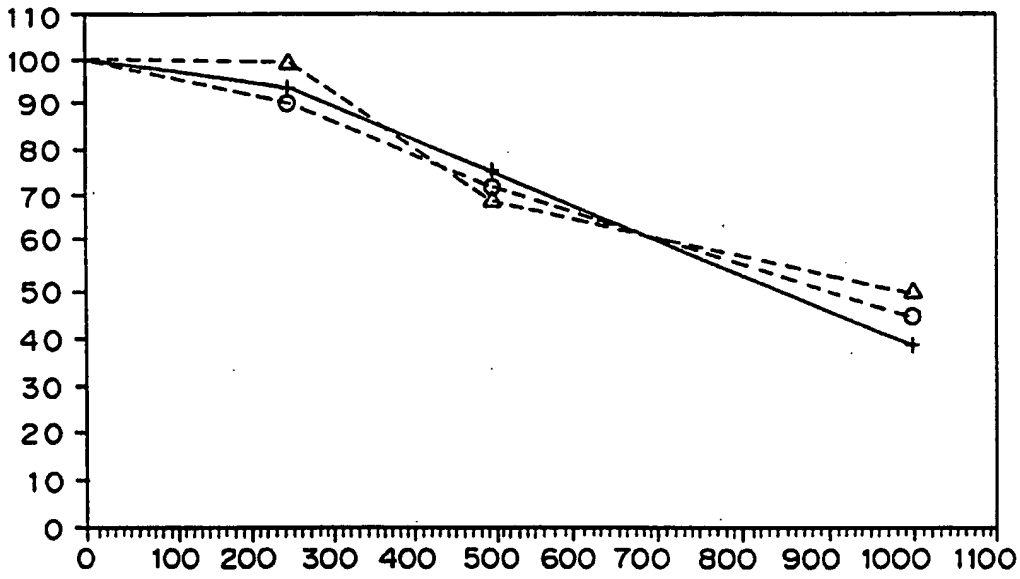

110
100
90
80
70
60
50
40
30
20
10
0
0 100 200 300 400 500 600 700 800 900 1000 1100
Drug concentration (uM)

FIG. 2

FIG. 3

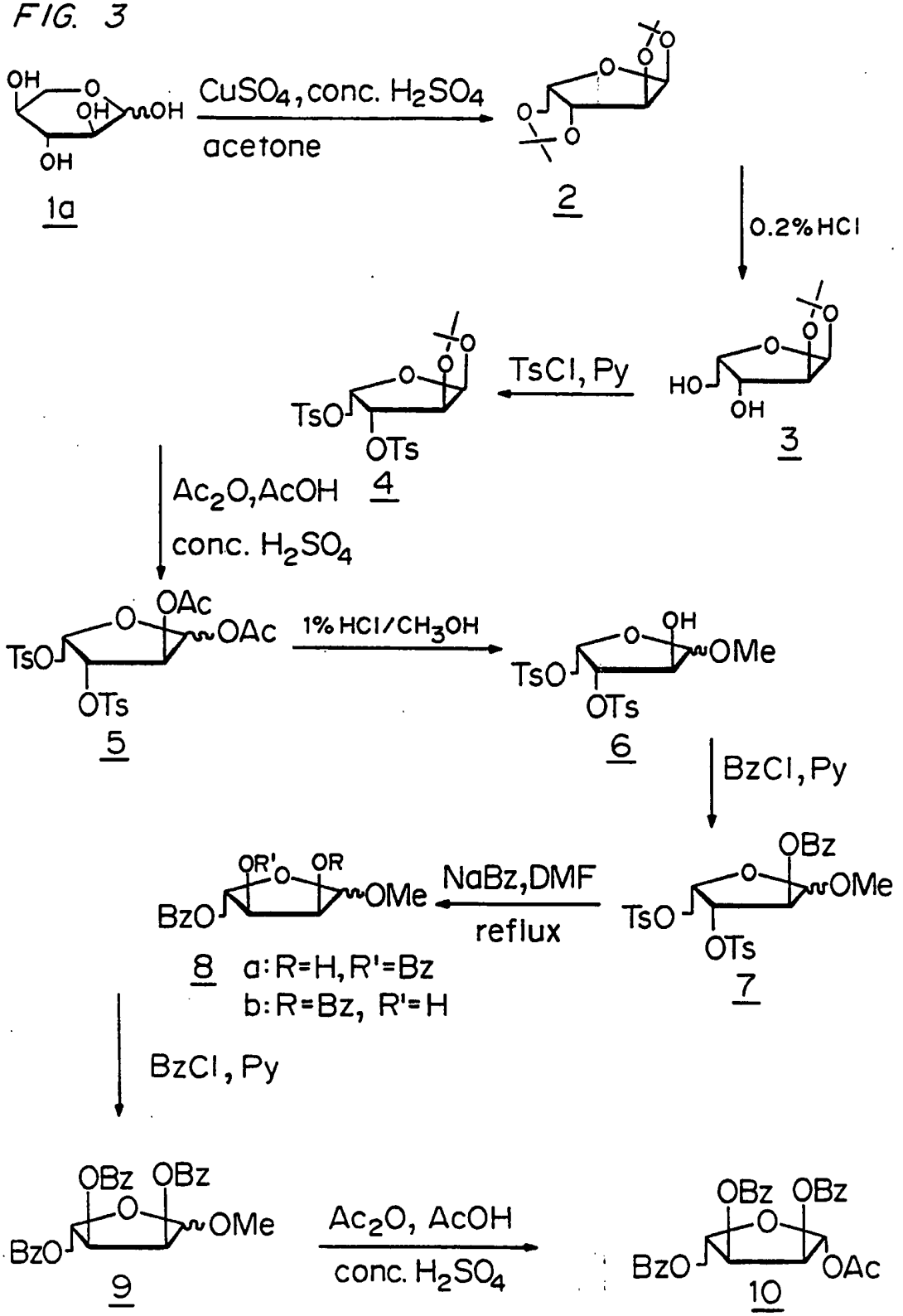

CuSO4, conc. H2SO4
acetone
1a
2
0.2% HCl
3
TsCl, Py
4
Ac2O, AcOH
conc. H2SO4
5
1% HCl/CH3OH
6
BzCl, Py
7
NaBz, DMF
reflux
8 a: R=H, R'=Bz
b: R=Bz, R'=H
BzCl, Py
9
Ac2O, AcOH
conc. H2SO4
10

FIG. 4

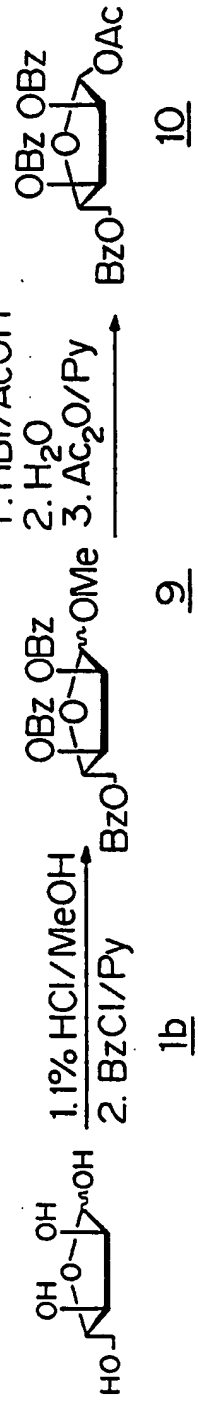
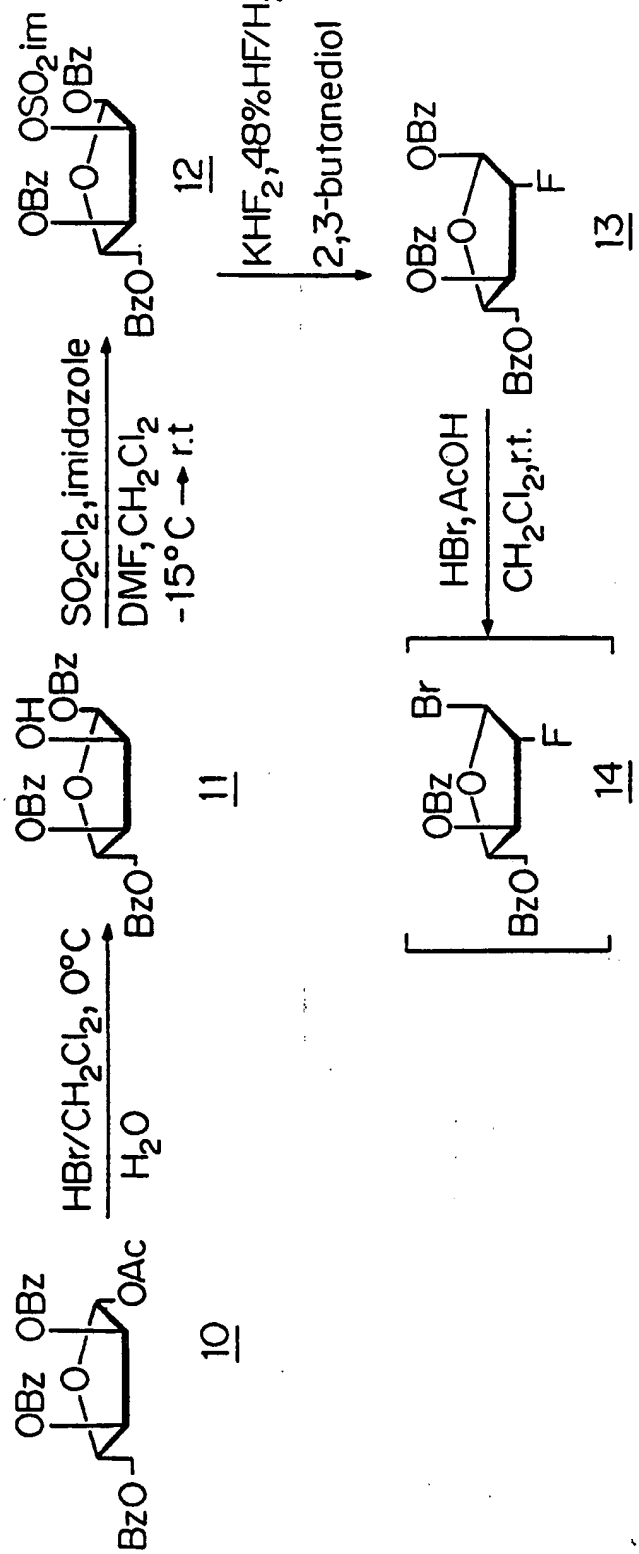

1.1% HCl/MeOH
2. BzCl/Py
1b
1. HBr/AcOH
2. H2O
3. Ac2O/Py
9
10

FIG. 5


HBr/CH2Cl2, 0°C
H2O
11
SO2Cl2, imidazole
DMF, CH2Cl2
-15°C → r.t
12
KHF2, 48% HF/H2O
2,3-butanediol
HBr, AcOH
CH2Cl2, r.t.
13
14

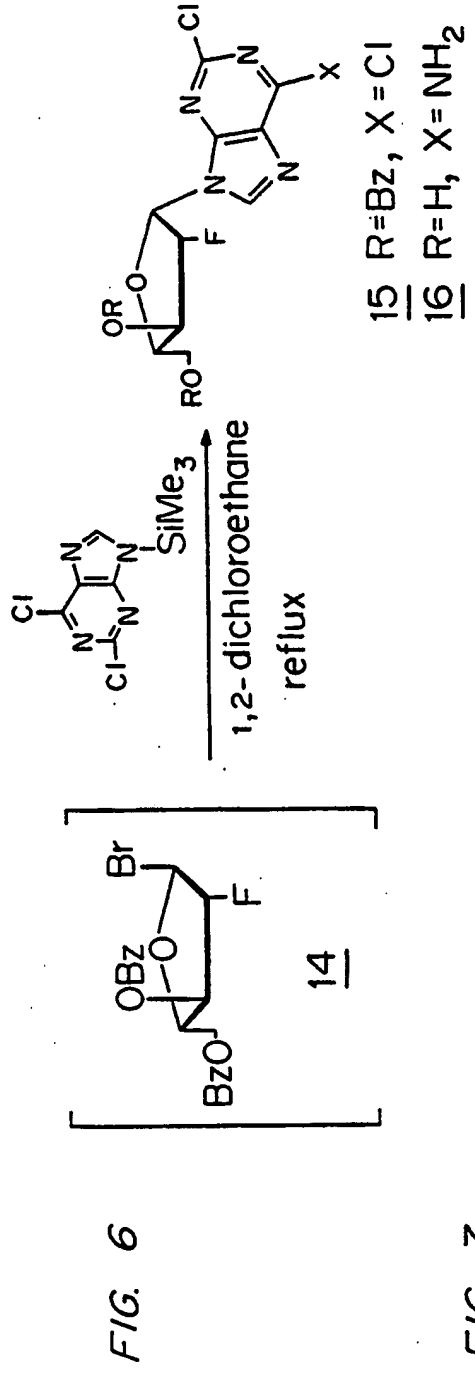

Br
F
OBz
BzO
14
1,2-dichloroethane
reflux
SiMe3
Cl
N
OR
RO
X
15 R=Bz, X=Cl
16 R=H, X= NH2

FIG. 6

Br
F
OBz
BzO
14
OSiMe3
Me3SiO
1,2-dichloroethane
reflux
O
NH
R
OR
RO
17. R=Bz, R'=CH3
18. R=H, R'=CH3
19. R=Bz, R'=C2H5
20. R=H, R'=C2H5
23. R=Bz, R'=1
24. R=H, R'=1

FIG. 7

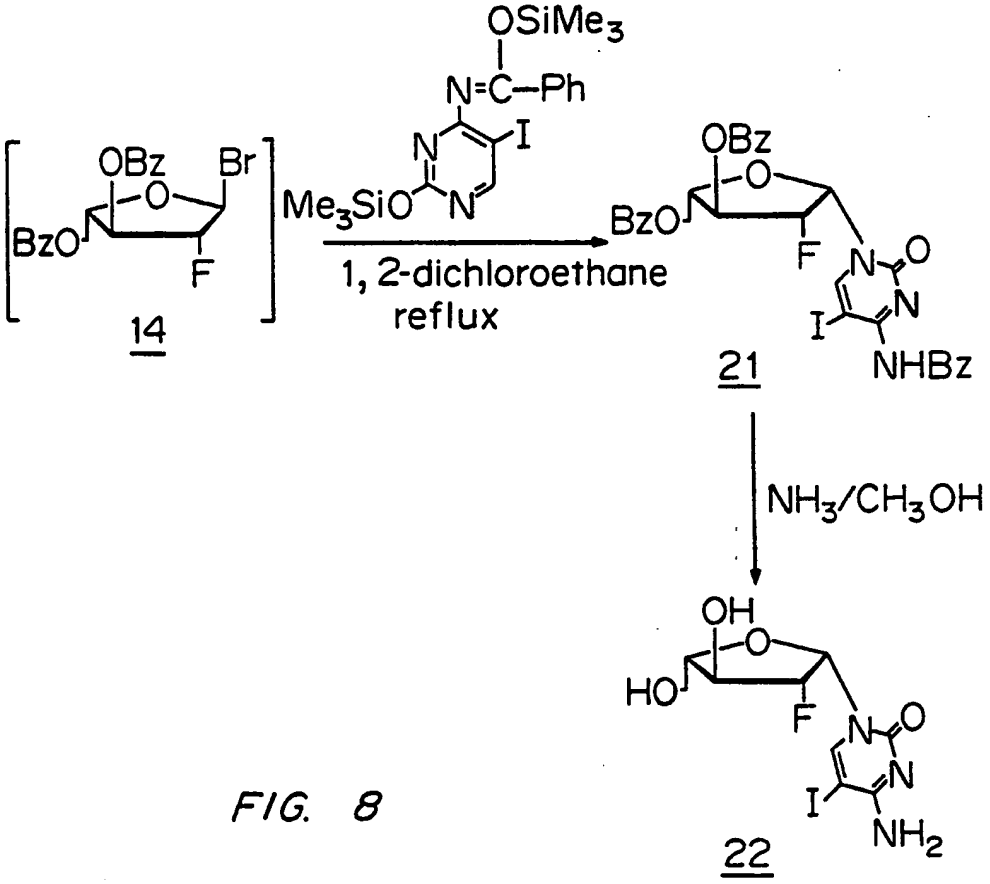

OSiMe3
N=C—Ph
I
N
Me3SiO
N
OBz
Br
O
BzO
F
14
1, 2-dichloroethane
reflux
OBz
O
BzO
F
N
O
N
I
NHBz
21
NH3/CH3OH
OH
O
HO
F
N
O
N
I
NH2
22

FIG. 8

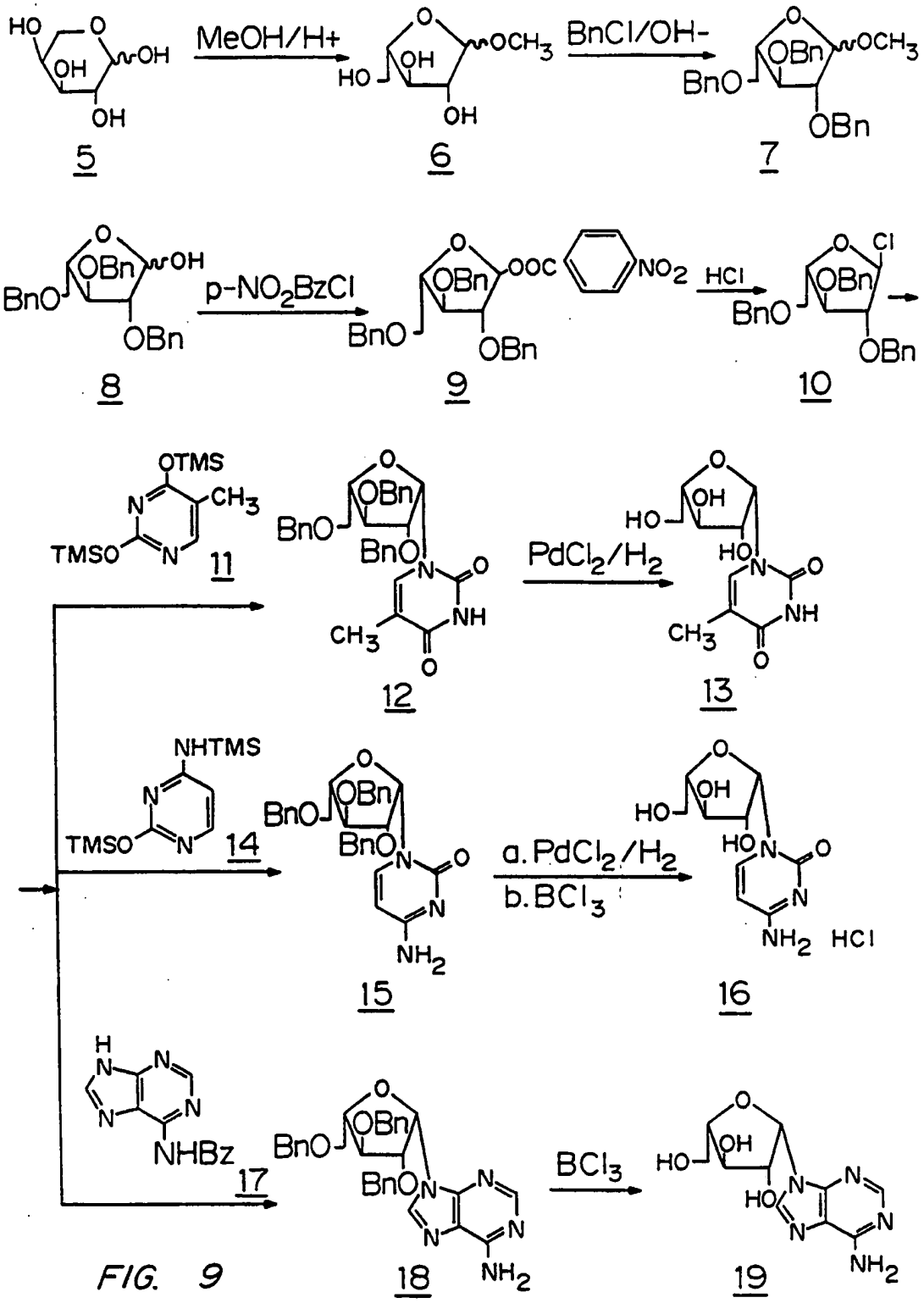

MeOH/H+
BnCl/OH-
p-NO2BzCl
HCl
PdCl2/H2
a. PdCl2/H2
b. BCl3
BCl3
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

FIG. 9

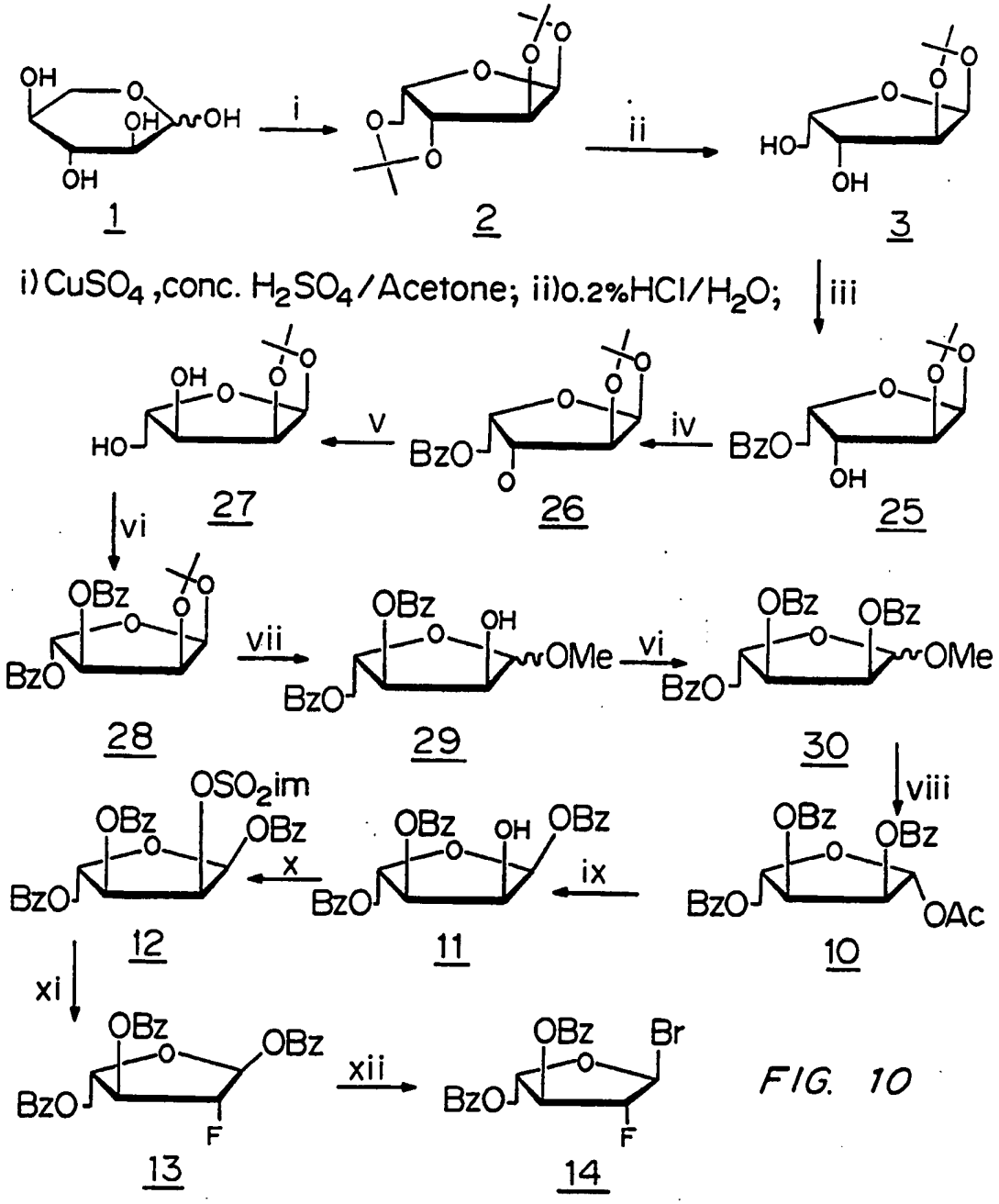

1
2
3
i) $CuSO_4$, conc. H_2SO_4/Acetone; ii) 0.2% HCl/H_2O; iii
25
26
27
28
29
30
10
11
12
13
14
iii) BzCl/Py/CH_2Cl_2, 0°C, 0.5hr. ; iv) PDC/Ac_2O/CH_2Cl_2, reflux;
v) $NaBH_4$/95% EtOH; vi) BzCl/Py; vii) 1% HCl/CH_3OH
viii) Ac_2O/AcOH/H_2SO_4; ix) HCl(g)/AcCl/CH_2Cl_2, 0°C, H_2O;
x) SO_2Cl_2, imidazole/DMF/CH_2Cl_2, -15° C to r.t.;
xi) KHF_2, 48% HF/H_2O/2,3-butanediol, N_2, reflux;
xii) HBr/AcOH/CH_2Cl_2, r.t.

FIG. 10

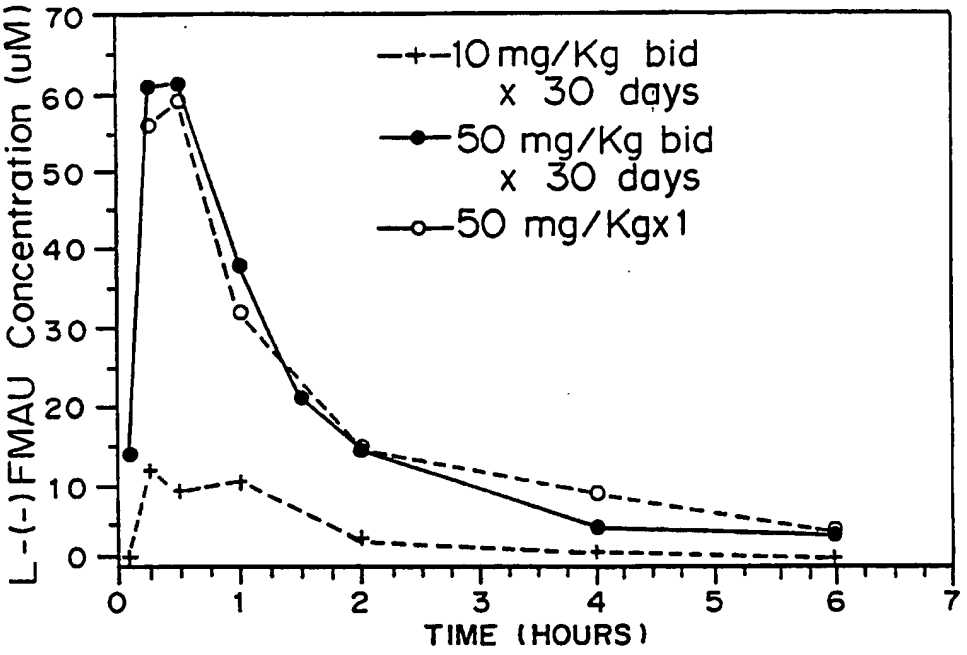

L-(-)FMAU Concentration (uM)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
TIME (HOURS)
10 mg/Kg bid x 30 days
50 mg/Kg bid x 30 days
50 mg/Kgx1

FIG. 11

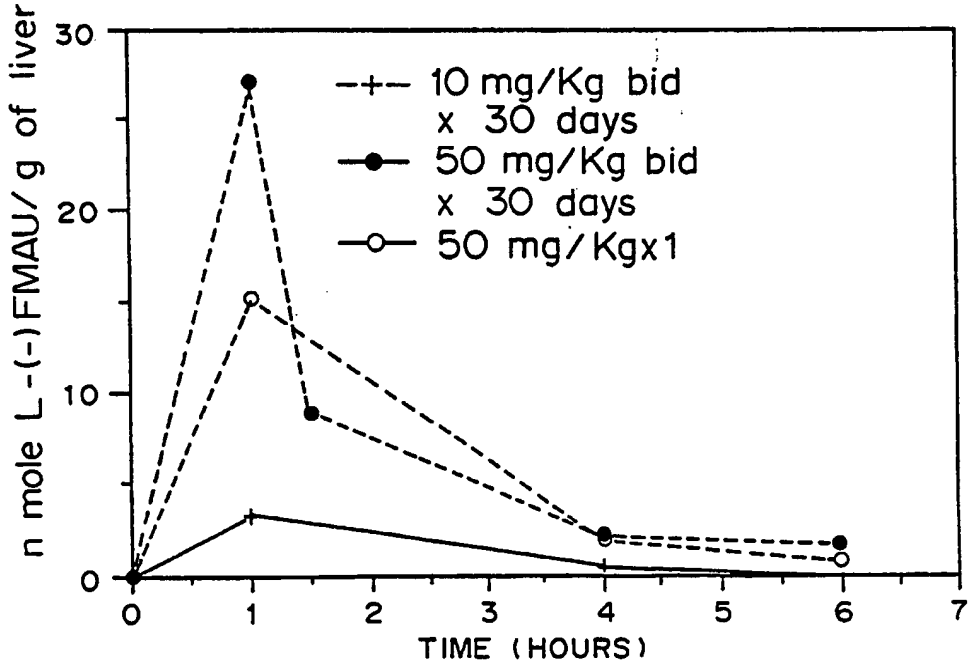
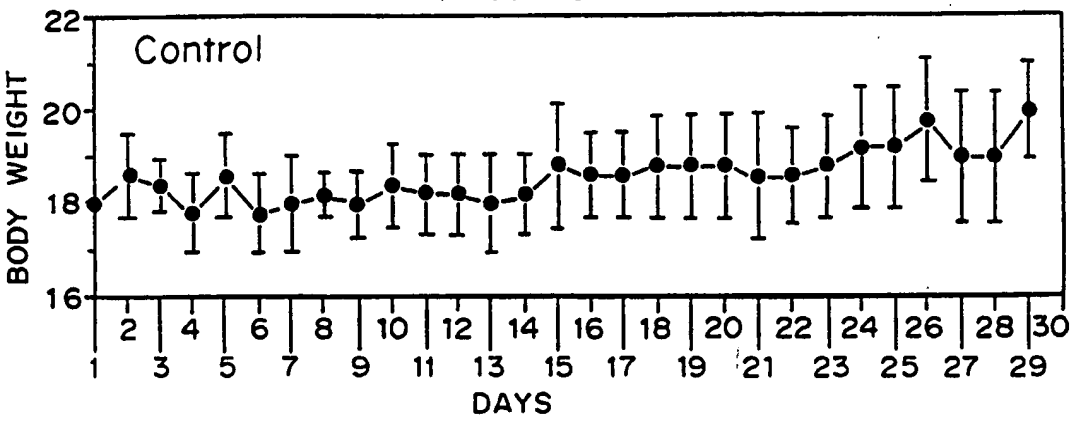
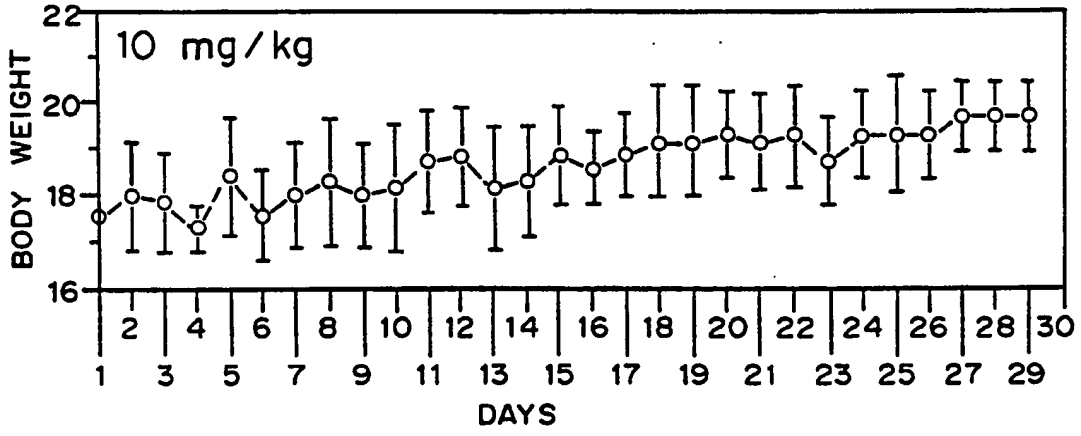
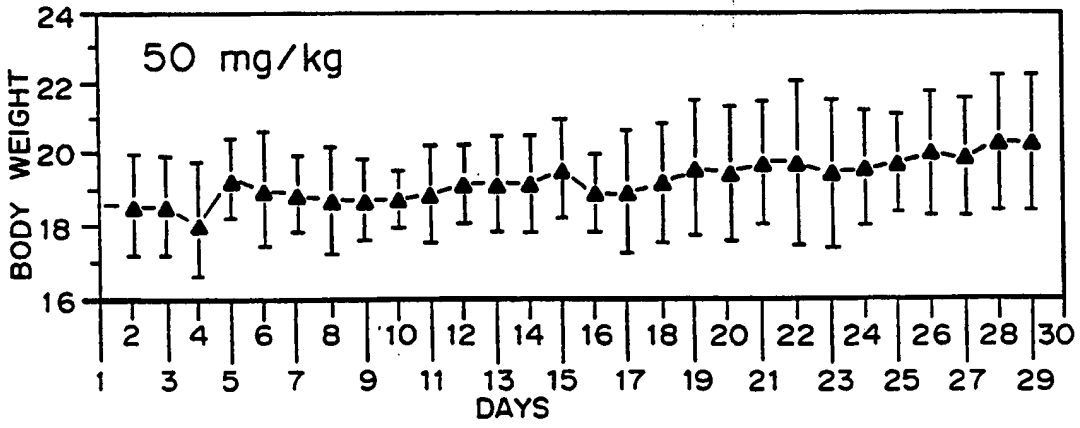

n mole L-(-)FMAU/g of liver
30
20
10
0
0
1
2
3
4
5
6
7
TIME (HOURS)
10 mg/Kg bid x 30 days
50 mg/Kg bid x 30 days
50 mg/Kgx1

FIG. 12

FIG. 13


Control
BODY WEIGHT
22
20
18
16
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
DAYS


10 mg/kg
BODY WEIGHT
22
20
18
16
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
DAYS


50 mg/kg
BODY WEIGHT
24
22
20
18
16
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
DAYS

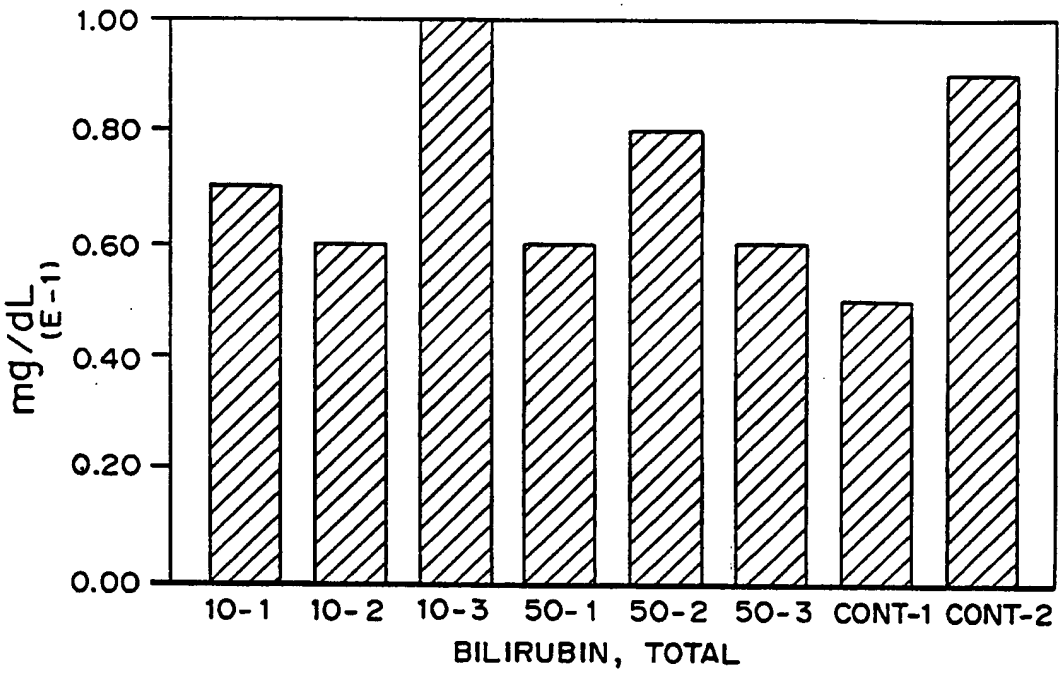

1.00
0.80
0.60
0.40
0.20
0.00
mg/dL (E-1)
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
BILIRUBIN, TOTAL

FIG. 14

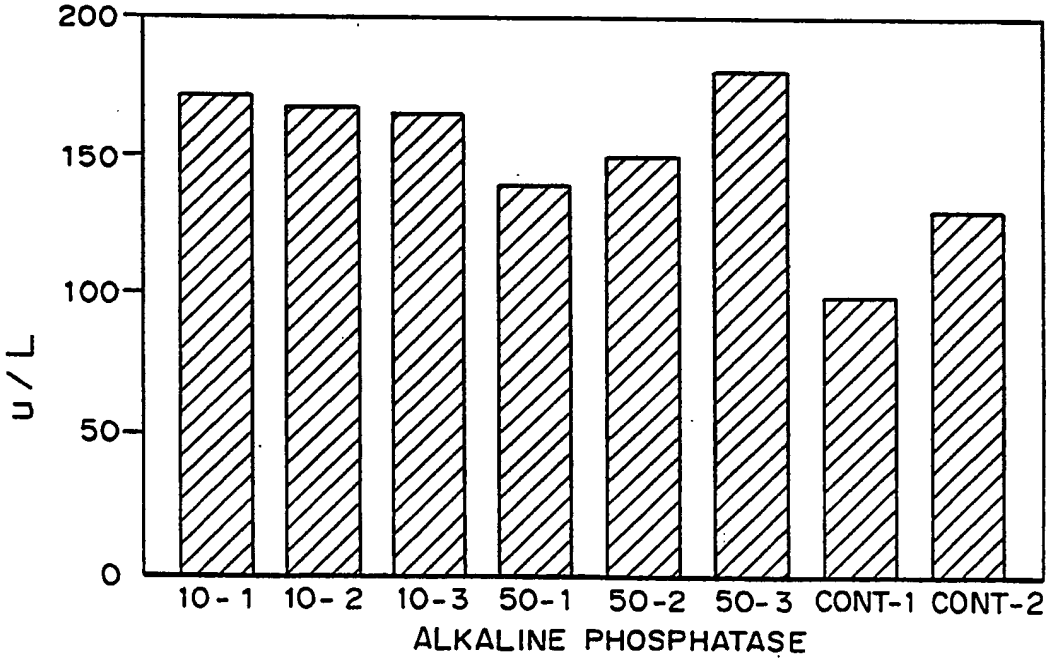

200
150
100
50
0
u/L
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
ALKALINE PHOSPHATASE

FIG. 15

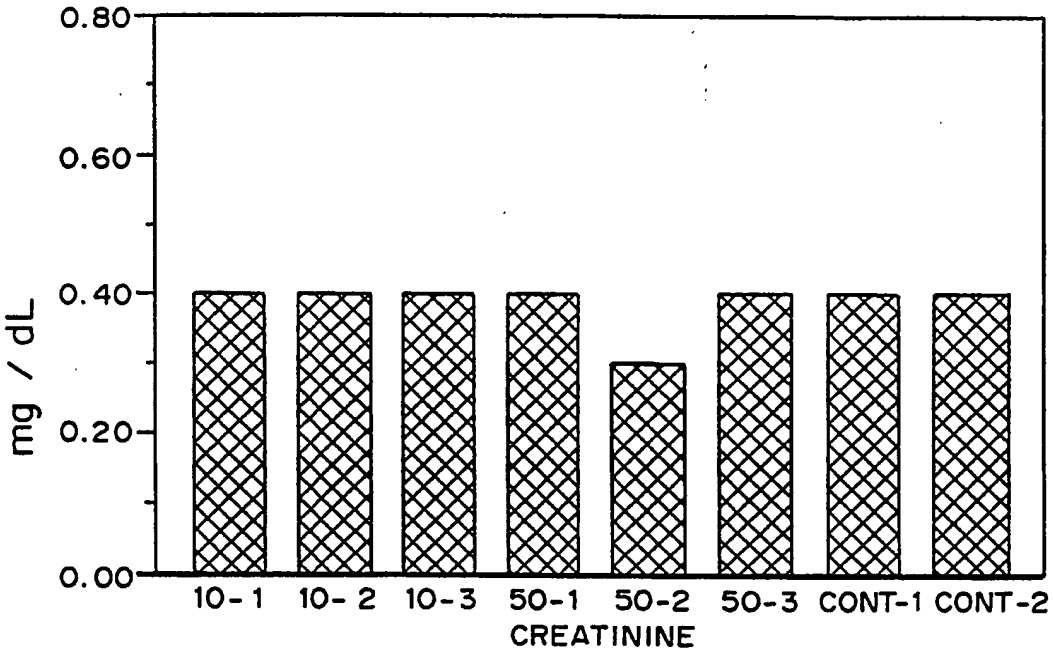

0.80
0.60
0.40
0.20
0.00
mg / dL
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
CREATININE

FIG. 16

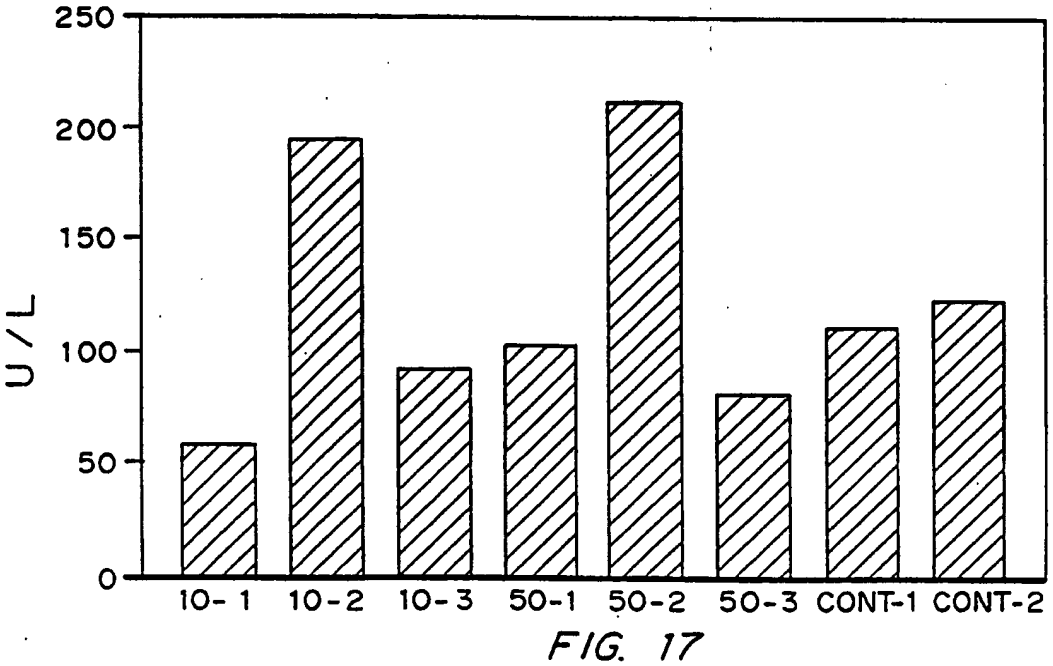

250
200
150
100
50
0
U / L
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2

FIG. 17

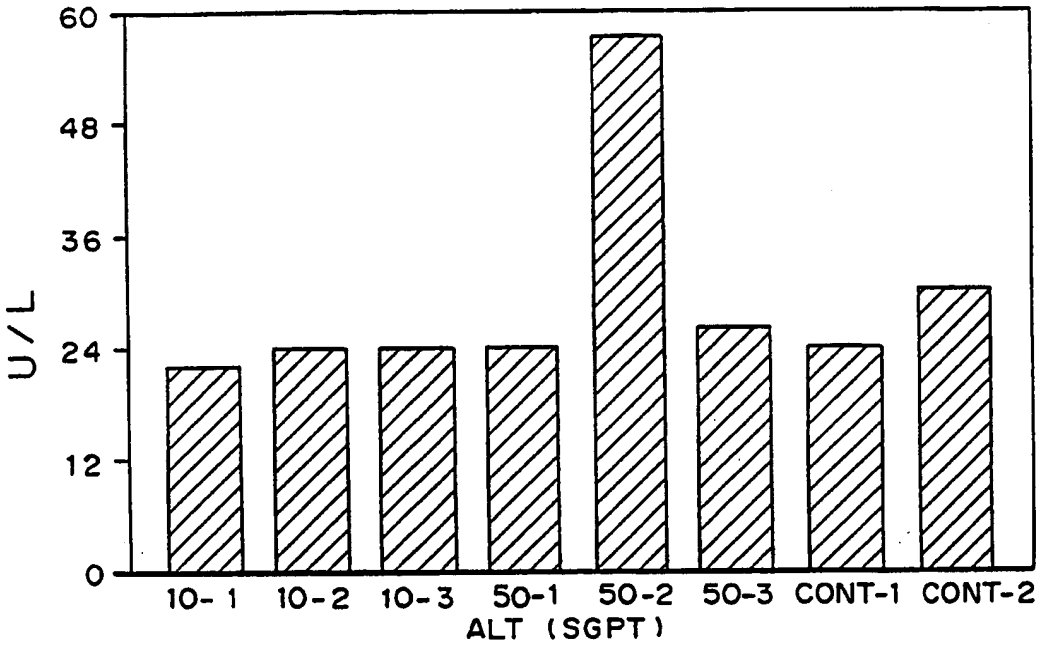

60
48
36
24
12
0
U/L
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
ALT (SGPT)

FIG. 18

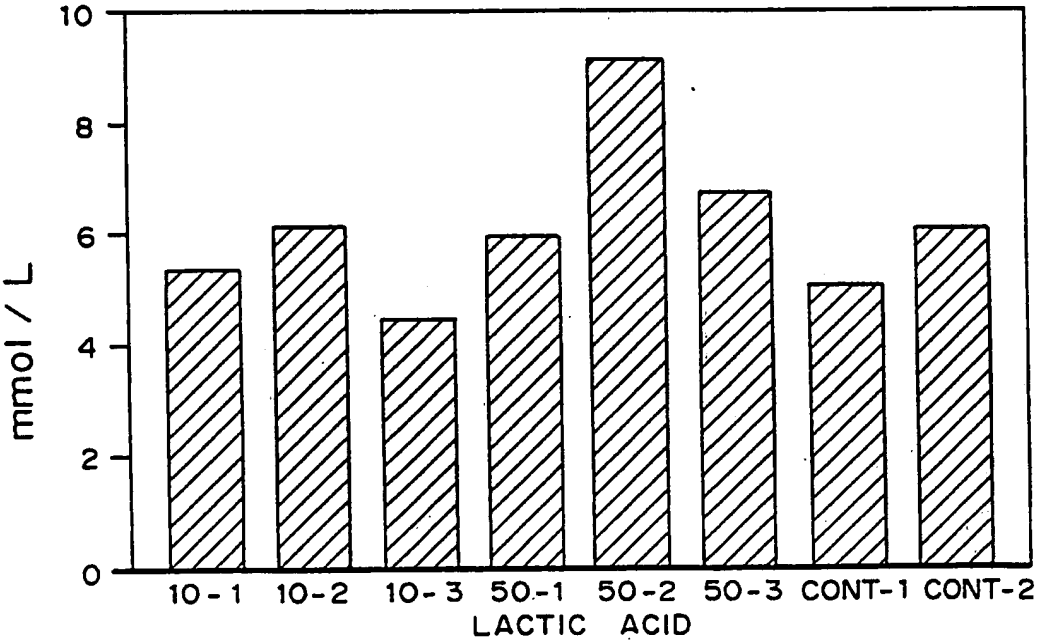

10
8
6
4
2
0
mmol / L
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
LACTIC ACID

FIG. 19

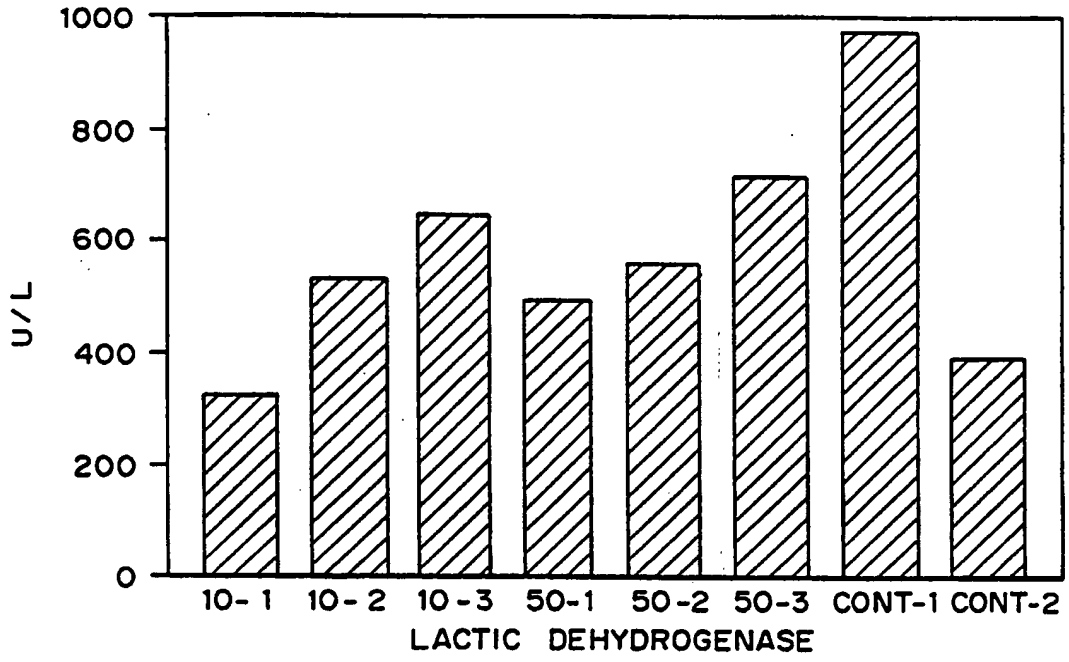

1000
800
600
400
200
0
U/L
10-1
10-2
10-3
50-1
50-2
50-3
CONT-1
CONT-2
LACTIC DEHYDROGENASE

FIG. 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01253

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 19/00, 19/06, 19/16; A61K 31/70
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/26.7, 26.8, 27.21, 28.5, 28.52, 28.54, 28.55; 514/ 49, 50, 51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 88/08001 (DATEMA ET AL.) 20 OCTOBER 1988, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 MAY 1995	Date of mailing of the international search report 09 MAY 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JAMES O. WILSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

international application No.
PCT/US95/01253

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/26.7, 26.8, 27.21, 28.5, 28.52, 28.54, 28.55; 514/ 49, 50, 51

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN files: Chemical Abstracts, BEILSTEIN, CHEMINFORMRX, GMELIN
search terms include: L(w) arabinofur?, (purin? or uridin? or cytosin? or pyrimid?), Herpes or EBV, pharmacol?,